THE UNIVERSITY *of* EDINBURGH

U N I V E R S I T Y O F E D I N B U R G H.

STUDY OF THE SEED MUCILAGE From

PLANTAGO LANCEOLATA.

By

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C O N T E N T S.

I N T R O D U C T I O N.

Acid polysaccharides, or polyuronides, may be defined as polysaccharides that contain one or more uronic acid units in their molecular structures. They have a frequent and wide occurrence in nature, much of the carbohydrate material in plants belonging to this group. It includes all pectic materials and plant gums, many plant mucilages, hemicelluloses, some bacterial polysaccharides, gel-forming substances (e.g., mucopolysaccharides) and alginic acid from algae. These polyuronides are to be found in water-soluble plant exudates and mucilages, as well as in water and alkaline extracts of most plant materials.

Previous to the last decade most of the research on the above group dealt with the composition of the substances; now, however, emphasis is shifting to a study of their structure and molecular size. Knowledge of the fine structure of those groups of polysaccharides which are more complex than starch or cellulose, in that they are built up of more than one type of sugar residue, is dependent on the elaboration of experimental techniques developed for the investigation of starch and cellulose. It requires also the elaboration of methods of the separation of closely

related sugar derivatives, and if there had been no other reasons than these, it would not have been surprising that attempts to investigate the detailed structures of members were of comparatively recent growth. Even so it seems probable that the development of further methods of attack will be necessary before a unique structure can be assigned to any one of these complicated polysaccharides. There are, however, further complications which arise from the colloidal character of such polysaccharides and close association in nature with other carbohydrate materials, which result in isolation and purification problems of unusual difficulty. Some of the so called hemicelluloses are of relatively simple structure in that they contain residues of one or two sugars or uronic acids only, while others, for example the plant gums, contain a wide variety of sugar residues linked together in the most diverse manner.(1)

The polyuronides are all stained by ruthenium red and all give the naphthoresorcinol test for a uronic acid.(2) They are hydrolysed by hot dilute solutions of strong acids to reducing sugars and aldobiuronic acids or free uronic acids. Plant gums and plant mucilages are present as salts, usually with calcium, magnesium and potassium as cations.

The pectic substances are a group of polyuronides which are of considerable botanical interest, as the young cells of the meristematic tissue(3) and

later the middle lamella of older tissue are believed to be pectic in nature. Indeed some of the earliest chemical investigations on pectin were instituted because of its botanical interest.(4) Fruits of all kinds contain considerable quantities of pectic materials the presence of which later confers upon jams their typical jellying property. These substances are apparently present in all plant tissues and are known also to occur in wood.(5) It must be understood, however, that the general term "pectin" is used to describe products which vary greatly in composition and possibly also in chemical constitution. Very different pectic products may be isolated from the same plant at different stages of growth. The difficult problem then arises as to whether pectins are physical mixtures of polysaccharides or whether they consist mainly of highly complex polysaccharides, the constitution of which varies from source to source. The problem is not yet completely solved but the former is favoured as the more acceptable explanation, pectic materials consisting generally of a mixture in varying proportions of at least three polysaccharides.

Originally it was considered that pectin was a polysaccharide built up of D-galactose, D-galacturonic acid and L-arabinose residues, as all the pectins so far isolated give on hydrolysis D-galactose, D-galacturonic acid, L-arabinose and methyl alcohol,

in varying proportions.

Pectins occur naturally in both soluble and insoluble forms. Soluble pectin occurs in plant juices and is particularly abundant in those juices which form jellies such as black-currant and gooseberry. Insoluble pectins tend to occur in the green part of the plant, in fruit and in root crops. This insolubility is apparantly due either to the presence of the pectin as its insoluble calcium or magnesium salt, or because it is combined with cellulose or some other insoluble polysaccharide of high molecular weight. The soluble form of pectin consists mainly of the methyl ester of pectic acid, together with galactan and araban.

The role of pectin in plant physiology is still only partly understood. It may be regarded as a cementing material, but it is obvious that the various types of pectic materials are able to perform in plants very varied biological functions.

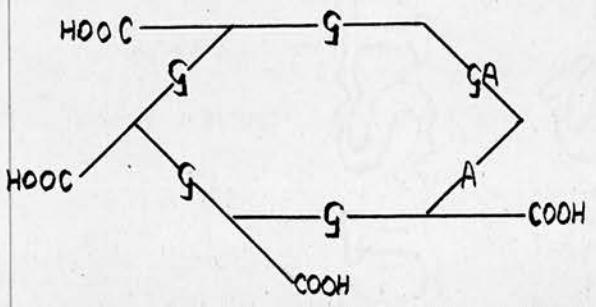
End group studies have not been used to determine the chain length, since only the partly

degraded pectin undergoes complete methylation.[6] Ultracentrifuge measurements show a lack of uniform particle size within any one pectin preparation and a variation in the average size of pectins from different sources. Values obtained by this method on pectins from various sources indicate that they have molecular weights varying from 25,000 to 50,000. If pectin were a simple polymerised galacturonic acid anhydride this maximum molecular weight would correspond to a chain length of approximately 280 D-galacturonic acid units; since, however, admixed arabans and galactans are present, the average chain length must be less than 280 units.[7]

Until recently the view forwarded by F. Ehrlich that pectin was a polysaccharide of low molecular weight was still generally held.[8] However, from the investigations of Baur and Link[9] and Schneider and Bock[10] it became apparent that pectins were to be regarded as mixtures of polysaccharides of high molecular weight, built on the same structural principles as cellulose and other polysaccharides.

F. Ehrlich was one of the first workers to attempt a detailed chemical investigation of pectin. He examined pectins from many sources and showed that the products were very similar in properties and reactions, but believed that pectins from different sources were chemical entities and not simply

differently proportioned mixtures of the same polysaccharides.[11] Nanji, Paton and Ling[8] were also of the opinion that the pectin molecule was one of low molecular weight, and suggested that the properties of pectic acid could best be explained on the basis of a six-membered ring containing D-galactose, L-arabinose and D-galacturonic acid residues.



Where

G = D-galacturonic acid

GA = D-galactose

A = L-arabinose

Pectic acid (Nanji, Paton and Ling)

More recent work on the chemical structure of pectic materials has been carried out by Hirst and Jones who achieved the separation of the araban, galactan and polygalacturonic acid portions of pectin, and did much to elucidate the molecular constitution of these respective polysaccharides.

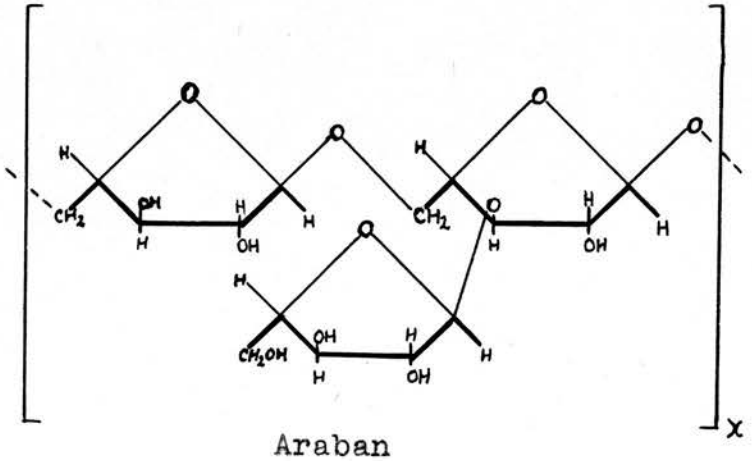
The first of the polysaccharides to be isolated in a high degree of purity was araban which was described by Ehrlich[12] in the course of his studies on pectins. It is characterised by its relatively high solubility in 70% alcohol and its ease of hydrolysis by boiling 0·01 N acid which converts it quantitatively

to L-arabinose. The ease of hydrolysis indicates that the polysaccharide is built up entirely of L-arabofuranose residues. The same araban is present in the pectin from citrus fruit, apple and peanut.(13)

On methylation the araban yielded a methylated derivative which was characterised by its high negative rotation ($[\alpha]_D$ - 180°) and by the extreme ease with which it underwent methanolysis. Hydrolysis of this product showed that the structure of the methylated polysaccharide was very different from that of pectic acid and the galactan with which it is associated. A fractionation of the resultant methylated arabinose derivatives(14) yielded equimolecular proportions of methyl 2:3:5-trimethyl-L-arabinoside, methyl 2:3-dimethyl-L-arabinoside and methyl 2-methyl-L-arabinoside. The dimethyl-L-arabinose residue might have existed in the polysaccharide either as a furanose or a pyranose form, but the high rate of hydrolysis of the araban in dilute acid was strong evidence that all the pentose residues were present in the furanose form. Whether the trace of unmethylated L-arabinose, which is also present, is an integral part of the molecule or is derived from incompletely methylated polysaccharide, or by demethylation of the arabinose fractions during methanolysis, has not yet been ascertained. For some time the exact identity of the monomethyl-L-arabinose was undecided, the methyl 3-methyl-L-arabinoside being

originally thought to be present in the hydrolysis products of the methylated araban,[14] since the amide from the syrupy hydrolysis product gave a positive "Weerman" test, indicating the presence of an α-hydroxy-amide. It has since been ascertained that this positive test was due to the presence of a small quantity of L-arabonamide in the syrupy amide, a trace of arabinose being present in the monomethyl arabinose fraction.[15] The decision between the possibility of the substance being methyl 2-methyl-L-arabinoside and methyl 3-methyl-L-arabinoside was reached after the synthesis of both derivatives of L-arabinose and their conversion into well characterised crystalline derivatives.[16]

The evidence so far available is not sufficiently detailed to enable a unique structure to be assigned to the repeating units of the araban molecule, but it is clear that a branched chain structure must be present. The general type of structure, which is one of the possible formulae in accordance with the experimental observations, is given below.



Araban

Arabans derived from apple and citrus pectins give on hydrolysis of the methylated derivatives approximately the same proportions of methyl 2:3:5-trimethyl-L-arabinoside, methyl 2:3-dimethyl-L-arabinoside and methyl 2-methyl-L-arabinoside. It is highly probable, therefore, that the arabans are structurally identical with one another. In this connection it may be noted that both Ehrlich and Schubert[12] and Gaponenkov[17] have investigated arabans obtained from various sources of pectin and have recorded physical constants with which the data obtained in this more recent work[16] are in reasonable agreement. It is considered likely, therefore, that all the samples of araban possessed the same type of constitution.

The only other araban which has been hitherto examined from the point of view of its chemical structure is the one present in gum tragacanth.[18] This araban has similar properties to those just described, but in addition to L-arabinose it contains a small amount of D-galactose. In the products of methanolysis methyl 2:3:5-trimethyl-L-arabinoside, methyl 2:3-dimethyl-L-arabinoside, β-methyl-L-arabo-pyranoside and methyl dimethyl galactosides were found.

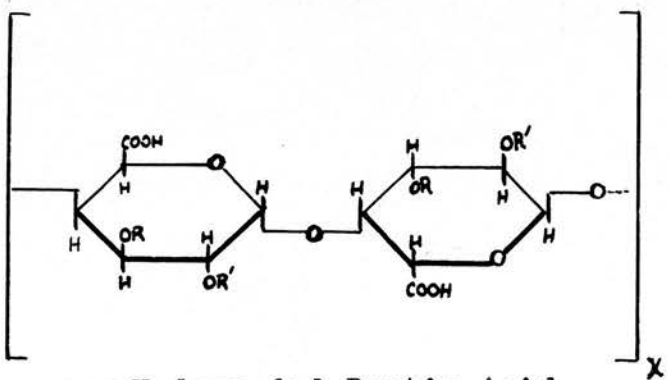
The constitution of the pectic acid fraction of pectin has been investigated by many workers, Saurez[19] and Ehrlich[20] being the first to show that pectic acid gave D-galacturonic acid on hydrolysis with acid.

The preparation by physical means of a pectic acid free from adsorbed araban and galactan is very difficult to achieve owing to the colloidal properties of the system. Separation by chemical methods is hindered by the instability of all three polysaccharides toward acids, and is further complicated by the instability of pectic acid toward alkali. Although pectic acid gives insoluble calcium and copper salts, this property cannot be used to separate araban and galactan free from pectic acid unless the latter is present to a small extent only. The removal of araban is a comparatively easy operation, but the isolation of a pectic acid free from both galactan and araban has only been achieved starting from a pectin initially low in galactan, or by the use of boiling methyl-alcoholic hydrogen chloride which preferably destroys the galactan and araban, but at the same time degrades the pectic acid,[9] the latter method being used by Baur and Link to prepare from several pectic acids, polygalacturonides containing 8 to 10 uronic acid residues. Henglein and Schneider[21] nitrated pectic acid under controlled conditions and examined the physical properties of the solutions of the resultant nitrate. Their results led them to the conclusion that pectic acid was a substance of high molecular weight, and that it consisted exclusively of condensed D-galacturonic acid units, and advocated in common with Baur and Link[9] a

1:4-glycosidic linkage as part of the pectic acid molecule. The first direct chemical evidence in favour of this was forwarded by Levene and Kreider[22] who oxidised a pectic acid with periodic acid and obtained L-(+)-tartaric acid after acid hydrolysis followed by bromine oxidation of the product. Isolation of this product does not decide whether the D-galacturonic acid is in the furanose form and linked 1:5 or in the pyranose form and linked 1:4. Since the polysaccharide is very resistant to hydrolysis by acidic reagents and shows a high positive optical rotation ($[\alpha]_D$ + 280°) it may be considered that pectic acid is built up of D-galacturonic acid residues in the pyranose form linked through hydroxyl groups C_1 and C_4 by α-glycosidic linkages - this conclusion being reached after a study of the hydrolysis products of methylated pectic acid by Beavan and Jones[23] and Smith.[24] This material which was relatively resistant to hydrolysis, on boiling with methyl-alcoholic hydrogen chloride under pressure was converted into methyl 2:3-dimethyl-D-galacturonoside methyl ester, identified after hydrolysis, oxidation and esterification as the methyl ester of 2:3-dimethyl-(+)-mucic acid. Neither of these groups of workers succeeded in isolating any methyl 2:3:4-trimethyl-D-galacturonoside and thereby obtaining some indication of the chain-length of the polyester. The reason for the absence of this end group is as yet unexplained.

The evidence so far indicates that degraded polygalacturonides practically identical in chemical and physical properties may be isolated from several different pectic acids, and there is strong evidence of pectic acids from several plant sources being of very similar, if not identical, constitution. Since only degraded pectic acids have so far been examined, there remains the possibility that undegraded pectic acid may consist of a main chain of D-galacturonic acid residues linked through carbon atoms 1 and 4, and that side chains of D-galacturonic acid residues in the pyranose or furanose form may be attached to this main portion of the molecule. Hydrolysis by the method of Link[9] would then result in the elimination of the more easily hydrolysed side chains and leave a degraded pectic acid in which the D-galacturonic acid residues are pyranose, linked through positions 1 and 4.

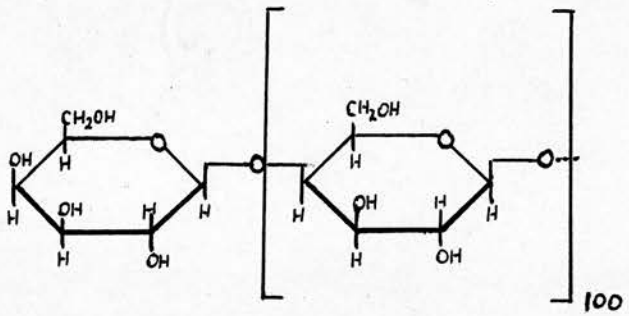
A possible formula for undegraded pectic acid is given below where side chains of D-galacturonic acid residues in the pyranose or furanose forms may be attached to one or both of the positions R and R'.



Undegraded Pectic Acid

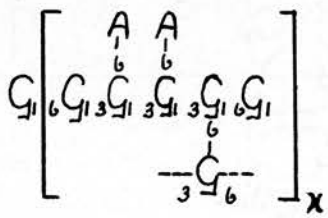
The galactan[25] associated with pectic acid and araban in pectin has been isolated in the pure state from one source only, the seeds of the white lupin (Lupinus albus). The isolation of this polysaccharide is facilitated by the low pectic acid content of the pectin component of the seeds and by the fact that araban can be removed by hydrolysis under mild conditions which are without significant effect on the galactan. This polysaccharide on methylation furnished a derivative ($[\alpha]_D$ - 20°) which was very resistant to acids and required long boiling with 5% methyl alcoholic hydrogen chloride to effect hydrolysis. The main product of hydrolysis of the methylated derivative was methyl 2:3:6-trimethyl-D-galactoside, identified after oxidation as the crystalline furanolactone. A small amount (approx. 1%) of methyl 2:3:4:6-tetramethyl-D-galactoside, identified as the crystalline anilide, was also present along with some unidentified methyl dimethyl-hexose, which may have been a methylated D-galactose derivative. The amount of methyl 2:3:4:6-tetramethyl-D-galactoside indicated a repeating unit of about 120 monomeric units in the galactan molecule. The low negative rotation of the methylated polysaccharide taken in conjunction with the relative stability of the polysaccharide to methyl-alcoholic hydrogen chloride and the isolation of some methyl 2:3:4:6-tetramethyl-D-galactoside from the products of hydrolysis, indicates

very strongly that the galactan consists of a chain of some 120 galactopyranose units united to one another by 1:4-β- links. The presence of small amounts of other sugars cannot be excluded from the evidence so far obtained.



Galactan from Pectin.

Only one other naturally occurring galactan has been examined in any great detail; this is the ε-galactan of the larch.[26] It contains D-galactopyranose and L-arabofuranose units and is a branched chain polysaccharide[27][28] very different from the galactan isolated from pectin. Although pectic materials are known to be present in wood, it is not clear whether this ε-galactan is a component of wood pectin, and in view of the wide difference in structure between this galactan and the one associated with pectin in lupin seeds, further investigation of this problem is to be awaited with interest.



Where

G = D-galactopyranose residues.

A = L-arabofuranose residues.

Possible formula for the repeating unit of ε-galactan from larch (E.V. White).

From the evidence at present available of the structural chemistry of pectic materials it would appear that neither the pectic acid nor the araban components can be derived directly from galactan.(1) To account for such a transformation by oxidation and decarboxylation the simplest hypothesis is to suppose that the galactan is hydrolysed in the plant to the free sugar and the resultant D-galacturonic acid then converted to pectic acid. Similarly it must be supposed that the D-galacturonic acid produced either from pectic acid or by oxidation of D-galactose is decarboxylated, after which the L-arabinose molecules combine together in the furanose form to produce an araban. The presence of an α-glycosidic linkage in pectic acid and a β-glycosidic linkage in galactan rules out immediately any possibility of direct conversion of the one to the other by enzymic oxidation of the terminal $-CH_2OH$ groups.

If it were supposed that the hexose polysaccharide was produced first and then transformed into pectic acid and araban, at least two types of galactan, other than the one already isolated from pectic materials, would be required to account for the type of structure present respectively in pectic acid and araban. In this case we might expect to encounter other types of pectic acid and araban, whereas from the evidence available the pectic acid isolated from all samples of

pectic materials examined appeared to be the same, as is the case with the araban.

When a plant is injured, either mechanically or by the invasion of fungi, the damaged area is sealed off by the production of a gum, thus preventing an increase in the area of injury. The origin of the gum is uncertain, yet there is a striking uniformity in the structure of gums isolated from different trees of the same type. Plant gums are divided into two groups, water-soluble and water-insoluble, depending on whether they dissolve in water to give a sticky solution, or whether they adsorb a large quantity of water to give a gel. Such a division is satisfactory up to a point but leaves obscure the distinction between a water-soluble gum and a mucilage. Similar difficulties occur in the case of gel-forming substances which may give a sticky solution at some concentrations and a gel at a higher concentration. No clear line of demarcation exists.

Some insight into the molecular structure of the gums and mucilages can be obtained from a study of their acidic hydrolysis products. This operation results in the removal of the more labile sugar residues and leaves a more resistant portion (an aldobiuronic acid) which is composed of a uronic acid residue united through its reducing group to another sugar. The structure of the more stable aldobiuronic acid fraction

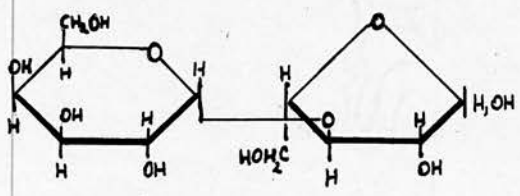
of the gum can be determined by the methods used in examining the reducing disaccharides.

The most important and best known water-soluble gum is gum arabic which finds many uses commercially as an adhesive, being obtained from trees and shrubs of the family Leguminosæ. This gum has been the subject of a series of detailed investigations by F. Smith, and notwithstanding the difficult and complicated nature of the problem, so much progress has been made that the main features of the structure of the repeating unit have been elucidated, and the possibilities remaining in structural detail have been considerably narrowed down.

The nature of the constituent sugars of gum arabic had already been investigated but some doubt remained as to the presence of L-rhamnose.(29) However, by autohydrolysis of the acidic polysaccharide Smith demonstrated the presence of L-arabinose, L-rhamnose and D-galactose. A portion of the galactose was isolated after partial hydrolysis of the gum in the form of a disaccharide, 3-D-galactosido-L-arabofuranose [I] the constitution of which was proved by the usual methods.(30) The constitution of the aldobiuronic acid which resulted from further hydrolysis of the degraded polysaccharide had already been determined as 6-D-glucuronosido-D-galactose [II],(31) hydrolysis of the octamethyl ether giving 2:3:4-trimethylglucuronic acid

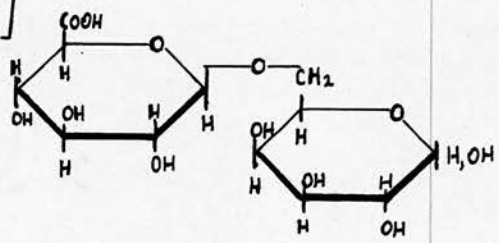
and 2:3:4-trimethyl-D-galactose.

[I]



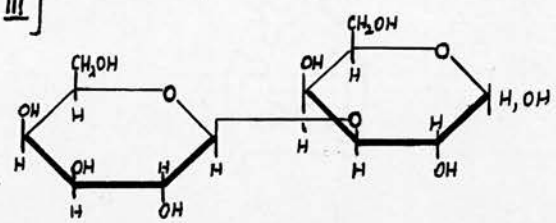
3-D-galactosido-L-arabofuranose.

[II]



6-D-glucuronosido-D-galactose.

[III]



3-D-galactosido-D-galactose.

Prolonged autohydrolysis of the degraded gum gave small quantities of a disaccharide 3-D-galactosido-D-galactose [III], the constitution of which was proved by hydrolysis of the completely methylated material and identification of the resulting sugars.(32) Degraded arabic acid (obtained by autohydrolysis of the gum and consisting of nine residues of D-galactose and three residues of D-glucuronic acid) was then methylated. From an examination of the sugars resulting from hydrolysis of the methyl derivative,(33) the presence of four end groups, one of D-galactopyranose and three

of D-glucuronopyranose, was demonstrated. The hydrolysis products included also 2:3:4-trimethyl-D-galactose (5 parts) and 2:4-dimethyl-D-galactose (3 parts). The isolation of these proved that all the galactose molecules were in the pyranose form and all linkages in the molecule were either 1:3 or 1:6. The possibilities of the structure of degraded gum arabic were further limited by the isolation under carefully controlled conditions of hydrolysis of a 6-β-D-glucuronosido-D-galactose from the methylated degraded arabic acid.(34)

One of the possibilities which illustrates the general type of structure is shown below [IV].

[IV]

Where

G = D-galactopyranose.

Glu = D-gluconic acid.

Degraded Gum Arabic.

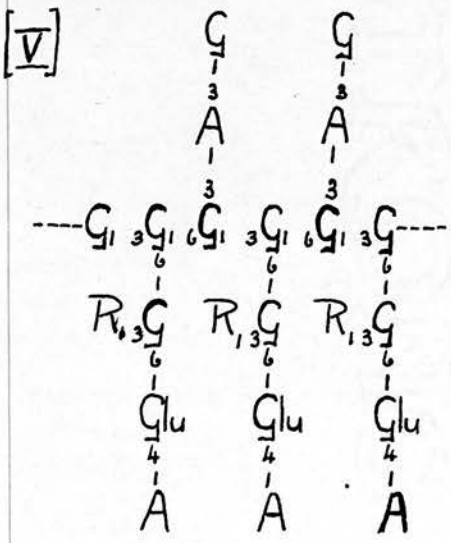
From a study of the products of hydrolysis of methylated arabic acid Smith was able to draw further conclusions as to the positions of the arabinose, rhamnose and galactose units. Hydrolysis gave 2:3:4-trimethyl-L-rhamnopyranose, 2:3:5-trimethyl and 2:5-dimethyl-L-arabofuranose, 2:3:4:6-tetramethyl and

2:4-dimethyl-D-galactopyranose and 2:3:4-trimethyl and 2:3-dimethyl-D-glucuronic acid.(35)

The identification of these products demonstrates the branched chain structure of arabic acid and also shows that these labile sugar residues, namely, L-arabinose, L-rhamnose and 3-galactosido-L-arabofuranose, which are liberated during the autohydrolysis of arabic acid, are joined to the nucleus of degraded arabic acid in the form of L-arabofuranose, L-rhamnopyranose and 3-galactosido-L-arabofuranose. In addition to the 1:3 and 1:6 linkages present in the arabic acid, the 1:4 linkage is also present, since the methyl 2:3-dimethyl-D-glucuronoside is one of the products isolated.

Many formulae varying only in detail are ascribable to the undegraded gum arabic [V] on the above evidence and still further work is required for the allocation of a definite detailed structure.

[V]



Where

G = D-galactopyranose.

Glu = D-glucuronic acid.

R = L-rhamnopyranose.

A = L-arabofuranose.

Gum Arabic (Smith).

Another water-soluble gum which has received some attention is Mesquite gum, being found as droplets exuding from the stem and branches of the mesquite tree (Prosopio juliflora). On hydrolysis, arabinose, galactose and glucuronic acid were originally found to be present.[36] Analysis of the gum acid agreed closely with a molecule composed of four units of L-arabinose, three of D-galactose and one of a methoxy-D-glucuronic acid. According to the nature of the hydrolysis the uronic acid was found to be joined to three, two and one molecule of galactose. In the latter case an aldobiuronic acid was obtained consisting of galactose and a methoxy-glucuronic acid. White[37] has more recently reported on the products obtained by methanolysis of the methylated mesquite gum. The uronic acid was isolated as methyl 2:3:4-trimethyl-D-glucuronoside, showing that in the original polysaccharide the uronic acid was present as a terminal unit, as were some of the L-arabinose residues, isolated as methyl 2:3:5-trimethyl-L-arabinoside. The branched chain nature of this gum is shown by the isolation of methyl 2:4-dimethyl-D-galactoside, indicating that the galactose units are pyranose and triply linked at 1, 3 and 6. The isolation of methyl 3:5-dimethyl-L-arabinoside shows that some of the arabinose units are linked through the 1:2 positions.

Gum tragacanth, which exudes from shrubs of

the genus Astralagus, has been shown by James and Smith[38] to consist of a complex mixture of a polyuronide (tragacanthic acid) a galacto-araban and a glycosidic substance probably of steroid character. Methylated tragacanthic acid methyl ester gave on methanolysis α methyl 2:3:4-trimethyl L-fucoside, methyl 2:3:4-trimethyl-D-xyloside, methyl 3:4-dimethyl-D-xyloside, methyl 2:3-dimethyl galacturonoside and a monomethyl galacturonoside methyl ester. The branched chain of the repeating unit is therefore terminated by L-fucopyranose and D-xylopyranose, while the D-galacturonic acid residues are believed to be pyranose also, and linked through C_1 and C_4 as in pectic acid. The highly branched nature of this gum is evident but differs from other plant gums in that it contains galacturonic acid and not glucuronic acid, while no acid residues appear as terminal groups.

The neutral polysaccharide associated with the tragacanthic acid was separated after methylation of the whole gum[38] and has been previously described.

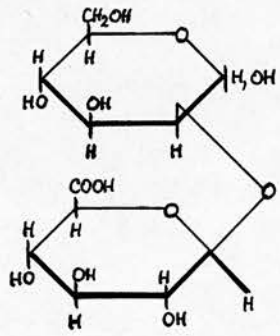
Another gum which has received considerable attention is damson gum, and structural investigations have been carried out by Hirst and Jones. From among the products of hydrolysis of the whole gum methylated by the thallous hydroxide method[39] they isolated 2:3:5-trimethyl-L-arabinose (8 parts), 2:3-dimethyl-L-arabinose (4 parts), 2:4:6-trimethyl-D-galactose (3 parts),

2:4-dimethyl-D-galactose (3 parts), 4-methyl-D-galactose (1 part), 2-methyl-D-galactose (1 part), 2:3:4-trimethyl-D-glucuronic acid (2 parts), 2:3-dimethyl-D-glucuronic acid (2 parts), together with unidentified derivatives of D-mannose and D-xylose.

Previous work on the hydrolysis of methylated degraded damson gum prepared by autohydrolysis[40] had resulted in the isolation of 2:3:4-trimethyl-D-xylose (3-4 per cent), 2:3:4:6-tetramethyl-D-galactose (1 part), 2:3:4-trimethyl-D-galactose (1 part), 2:4:6-trimethyl-D-galactose (1 part), 2:4-dimethyl-D-galactose (1 part), 2:3:4-trimethyl-D-glucuronic acid (1 part), 2:3-dimethyl-D-glucuronic acid (1 part), and a partly methylated mannose. Since 2:3:4:6-tetramethyl-D-galactose and 2:3:4-trimethyl-D-galactose do not appear in the hydrolysis products of the methylated whole gum it may be inferred that the L-arabinose side chains, which are probably all furanose in form, are attached to positions C_6 and C_3 in these galactose residues which give rise to the above mentioned sugars in the methylated degraded gum. During the preparation of the degraded gum by autohydrolysis all the L-arabinose residues are removed. From the fact that 2:3:5-trimethyl and 2:3-dimethyl-L-arabinose residues are produced on hydrolysis of the methylated gum in the ratio of 2:1 it follows that there must be two side chains on each repeating unit which consist of L-arabinose only, one

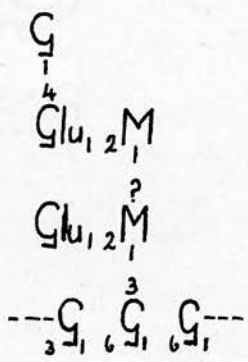
side chain consisting of a single L-arabofuranose residue and the other of two such molecules linked through C_1 and C_5. From these facts and from the observation that the aldobiuronic acid in damson gum consists of 2-D-glucuronosido-D-mannose [I] some idea can be obtained of the type of structure present in the damson gum molecule. Neglecting, for the moment, the small proportion of xylose the role of which is unknown, one of the many possible structures for degraded damson gum is given below [II] .

[I]



2-D-glucuronosido-D-mannose.

[II]



Where

G = D-galactopyranose.

Glu = D-glucuronic acid.

M = D-mannopyranose.

It is obvious that until detailed information concerning the mannose and xylose residues is obtained it will not be possible to indicate more than an outline of the type of structure involved.

Butler and Cretcher[41] examined a gum obtained from the wild cherry trees of Arizona and came to the conclusion that it was a polysaccharide consisting of L-arabinose (8 molecules), D-xylose (6 molecules), D-galactose (3 molecules) and D-glucuronic acid (2 molecules). The gum examined by Jones[42] is fundamentally different from that described by the American authors, and Jones has found that cherry gum closely resembles damson gum in its physical properties, but differs from it in its chemical constitution. He had shown[42] that cherry gum contains the following sugars in the approximate proportions indicated: D-glucuronic acid (1 part), D-mannose (1 part), D-galactose (2 parts), L-arabinose (6 parts), and D-xylose (ca. 1·5%). Autohydrolysis of the acidic gum resulted in the removal of practically all the pentose, leaving a degraded cherry gum which closely resembled the degraded gum from the damson. On further hydrolysis this gives two molecular portions of D-galactose together with an aldobiuronic acid identified as 2-D-glucuronosido-D-mannose identical with the aldobiuronic acid from damson gum.

Methylated cherry gum gives on methanolysis a complex mixture of sugars among which the following have been identified:[43] 2:3:5-trimethyl-L-arabinose, 2:5-dimethyl-L-arabinose, 2:4:6-trimethyl-D-galactose, 2:4-dimethyl-D-galactose, 2:3:4-trimethyl-D-glucuronic

acid and 2:3-dimethyl-D-glucuronic acid. Derivatives of D-mannose and of D-xylose must be present but their isolation has not yet been accomplished. Owing to the difficulty in identifying and separating quantitatively the various glycosides, it was not found possible to give a precise quantitative estimate of the various sugar derivatives formed on hydrolysis of the methylated polysaccharide. Hence no attempt was made to give a unique formula for cherry gum at this stage. It had already been shown that 2-D-glucuronosido-D-mannose is part of both the damson and cherry gum molecules,[40] and it is obvious that both polysaccharides possess other points of similarity. For example, D-galactose units which are linked through positions C_3 and C_1 and C_6 , C_3 and C_1 occur in both; in addition, both molecules contain terminal L-arabinose and D-glucuronic acid units, and a D-glucuronic acid unit linked through C_1 and C_4 is common to both gums. A point of difference, however, is that in cherry gum L-arabinose residues occur which are linked through C_1 and C_3 (cf. gum arabic[35]), whilst in damson gum the linkage is through C_1 and C_5. It seems that cherry gum is built up on the same lines as damson gum and gum arabic with probably a main chain of D-galactose units to which are attached side chains of aldobiuronic acids and pentose residues.

Continuing their studies on gums, Hirst and

and Jones[44] have examined the gum exuded by the egg or yellow Pershore plum tree (family Rosaceæ). This polysaccharide has been shown to consist of L-arabinose (3 parts), D-xylose (1 part), D-galactose (3 parts) and D-glucuronic acid (1 part). The aldobiuronic acid which is present in the portion of the gum molecule more resistant to acidic hydrolysis is 6-D-glucuronosido-D-galactose, identical with the aldobiuronic acid component in gum arabic. Owing to the difficulty encountered in isolating the aldobiuronic acid component of the gum in quantitative yield, the possibility remains that some aldobiuronic acid other than 6-D-glucuronosido-D-galactose may be a component of the gum molecule.

Further insight into the structure of the stable portion of the gum molecule was derived from a study of the hydrolysis products obtained from the methylated derivative of the degraded gum.[45] The following sugars were identified amongst the products of hydrolysis of the methylated degraded egg-plum gum: 2:3:4:6-tetramethyl-D-galactose, 2:4:6 and 2:3:4-trimethyl-D-galactose, 2:4-dimethyl-D-galactose and 2:3-dimethyl-D-glucuronic acid. A partly methylated aldobiuronic acid, 6-(2:3-dimethylglucuronosido) 2:4-dimethyl-D-galactose, was also found amongst the products of hydrolysis.

The degraded polysaccharide is therefore not

identical structurally with the degraded polysaccharide obtained from gum arabic, although on hydrolysis both these degraded gums give D-glucuronic acid (1 part) and D-galactose (3 parts).

As in the case of damson gum a satisfactory quantitative estimation of the sugars present in the methylated degraded molecule could not be obtained owing to the complex nature of the mixture which resulted on hydrolysis, and therefore a unique structure could not be assigned. It will be observed, however, that D-galactose is the major component of the degraded gum and that the 1:3 and 1:6 linkages are again encountered as in the main chains of gum arabic, damson gum and cherry gum.

The substance extracted from plants by 4% caustic soda after preliminary extractions with water and 0·5% ammonium oxalate (to remove pectic substances) is described as a hemicellulose, the name being proposed by Schulze[46] who considered this substance was related to cellulose. Schulze showed that the substance extracted with dilute alkali and precipitated by acid was much less resistant than cellulose to hydrolysis, but he believed that it might be an intermediate precursor of cellulose. The elucidation of the structure of cellulose and the analysis of hemicellulose have shown that there is no foundation for this belief. Separation of the hemieellulose from

co-extracted cellulosan and lignin presents considerable difficulties and there is some doubt whether a hemicellulose entirely free from traces of cellulosan has yet been prepared. (The term cellulosan being used to describe the glucosans and xylans associated with natural cellulose).

The constitution of hemicelluloses has seldom been approached by classical methods. O'Dwyer[47] in 1928 methylated oakwood hemicellulose with dimethyl sulphate and sodium hydroxide, but did not proceed beyond this point. A study of the constitution of the hemicellulose of Iceland moss (Cetraria Islandica), a non-lignified tissue, has been carried out more recently by Granichstadten and Percival.[48] Hydrolysis of the polysaccharide gave a mixture of sugars containing glucose (89%), galactose (8%) and mannose (3%). A uronic acid, probably D-glucuronic acid (5%), was also identified. Methylation yielded a product (OMe, 41-43%) which was separated by precipitation from chloroform with light petroleum into four fractions, A, B, C and D, and since these fractions differed significantly in rotation and viscosity, they were examined individually. Methanolysis and fractionation of the products gave an end-group of D-galactopyranose in A, and end-groups of both galacto and glucopyranose in B, while no end-groups were detected in C and D. The main fraction of each hydrolysate consisted of a mixture of trimethylglucoses;

2:3:4, 2:3:6, 2:4:6 and3:4:6-trimethylglucoses were identified. The end-group fractions in A and B proved to be 2:3:4:6-tetramethyl-D-glucose and 2:3:4:6-tetramethyl-D-galactose. To interpret these results satisfactorily is difficult. The lack of homogeneity of the methylated hemicellulose may indicate a mixture of polysaccharides. Differences in rotation would indicate different linkages in these individual polysaccharides, while variations in viscosity would suggest a difference in chain length. It is obvious that the main chain is constituted of D-glucose residues united by a variety of linkages, viz., 1:2, 1:3, 1:4 and 1:6. Furthermore, the chain must be branched with at least two branches terminated by D-glucopyranose and D-galactopyranose units respectively. That a single polysaccharide would contain so many different linkages is highly improbable. Two alternative interpretations are submitted by Granichstadten and Percival:- (1) The hemicellulose is a mixture of at least four polysaccharides in each of which the linkage is uniform (1:2, 1:3, 1:4 or 1:6). (2) It may be a mixture of polysaccharides in which mixed linkages exist. In their methanolysis the authors were unable to detect a uronic acid. The 5% methyl-alcoholic hydrogen chloride used may have been sufficient to cause degradation of this component.

The polyuronide hemicellulose of Phormium tenax, a lignified fibre, has been subjected to methylation studies by McIlroy, Holmes and Mauger,(49) the polysaccharide giving on hydrolysis D-xylose and D-glucuronic acid, the latter as a component of an aldobiuronic acid. Methylation of the hemicellulose gave a homogeneous product (OMe, 36·8%) which yielded on methanolysis methyl 2:3:4-trimethyl-D-xyloside (11% as end-grouping), methyl 2:3-dimethyl-D-xyloside (main fraction) and a methylated aldobiuronic acid methyl ester which was separated as the barium salt. A mutorotational change in an upward direction indicated a predominance of β-linkages in the main xylose chain. Nitric acid oxidation of the aldobiuronic acid gave xylohydroxy-dimethoxy-glutaric acid, 2:3-dimethyl-γ-saccharo-lactone, 2:3:4-trimethyl-δ-saccharolactone and dimethoxy-succinic acid.

From the above evidence it is concluded that Phormium hemicellulose is constituted of a main chain containing 9-10 xylopyranose residues united by 1:4-β-glycosidic linkages, terminated at the reducing end by an acid of complex structure.

The structure of the polyuronide mucilages has been less thoroughly studied than that of the plant gums, the acidity of which, it will be noted, is due - except in the case of gum tragacanth - to D-glucuronic acid, and it would appear that one of the differences

frequently observed between gums and mucilages is that the acidity of the latter is due usually to D-galacturonic acid.

In 1913 Neville[50] had realised that the mucilage from flaxseed yielded sugars on hydrolysis and thought it possible that the mucilage was the salt of a complex acid akin to the gums; however, in his work he was unable to characterise any acid portion. No other reference to work on plant mucilages is to be found until 1930 when Anderson and his co-workers began to tackle the subject systematically. Anderson and Crowder[51] at first examined flaxseed mucilage, identifying among the hydrolysis products, L-galactose, D-xylose, D-glucose and L-arabinose, along with an aldobiuronic acid made up of D-galacturonic acid and L-rhamnose.

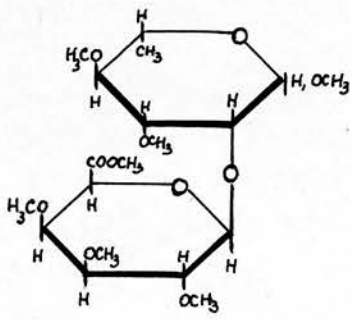
In 1934 Anderson[52] examined the mucilage obtained from the inner bark of the slippery elm (Ulmus fulva), isolating an aldobiuronic acid consisting of L-rhamnose and D-galacturonic acid, and in the hydrolysis liquid proved the presence of D-galactose. An investigation[53] on the nature of the mucilage obtained from light psyllium seed (Plantago psyllium) followed, and from the products of hydrolysis D-xylose, L-arabinose and D-galacturonic acid were identified. The aldobiuronic acid was characterised as an arabinose-galacturonic acid combination.

In all the above-mentioned work on mucilages

characterisation of the sugars present was all that had been attempted, and a more complete understanding of the structural design of the mucilages had to await the introduction of methylation studies which have come to be regarded as the classical method of approach to such a problem.

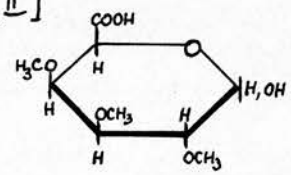
Further study of the mucilage from the bark of Ulmus fulva (slippery elm) has been carried out by Gill, Hirst and Jones.(54) These workers showed that hydrolysis of the polysaccharide with dilute acid yields D-galactose, L-rhamnose and an aldobiuronic acid identified as 2-D-galacturonosido-L-rhamnose, the structure following from the identification of the sugars isolated from the hydrolysis of the methylated aldobiuronic acid [I]. These sugars are 2:3:4-trimethyl-D-galacturonic acid [II], and 3:4-dimethyl-L-rhamnose [III].

[I]



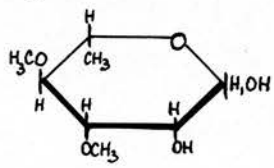
Methyl ester of 2(2:3:4-trimethyl-D-galacturonosido)-3:4-dimethyl-L-rhamnose.

[II]



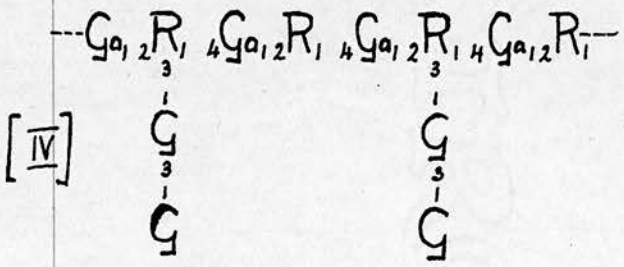
2:3:4-trimethyl-D-galacturonic acid.

[III]



3:4-dimethyl-L-rhamnose.

The aldobiuronic acid is identical therefore with the acid isolated from linseed mucilage[55] (Linum usitatissimum), and contains the comparatively rare 1:2 linkage which occurs in the aldobiuronic acid derived from damson and cherry gums. In a later publication on the mucilage from the bark of Ulmus fulva, Gill, Hirst and Jones[56] report the isolation of a protein-free polysaccharide. Methanolysis, followed by hydrolysis of the fully methylated mucilage, gave 2:3-dimethyl-D-galacturonic acid (4 parts), 2:3:4:6-tetramethyl-D-galactose (2 parts), 2:4:6-trimethyl-D-galactose (1 part), 2:3:6-trimethyl-D-galactose (1 part), 3:4-dimethyl-L-rhamnose (2 parts), 4-methyl-L-rhamnose (2 parts) and a trace of 2:3:4-trimethyl galacturonic acid. These products of hydrolysis of the methylated mucilage reveal the general structure but show that no unique formula for slippery elm mucilage can be advanced at this stage. One possible structure of several which fit the known facts is given below [IV].



[IV] Ulmus fulva.

Where

Ga = D-galacturonic acid.

G = D-galactopyranose.

R = L-rhamnose.

Nelson and Percival[57] have made a study of the mucilage obtained from dark psyllium seeds (Plantago arenaria) and from the products of hydrolysis identified D-xylose (70%), L-arabinose (9·5%), D-galactose (3·3%) and an aldobiuronic acid (13%) composed of D-galacturonic acid and D-xylose. Hydrolysis of the aldobiuronic acid at 120° with sulphuric acid (4%) followed by neutralisation with barium carbonate yielding a glass, from which extraction with alcohol gave D-xylose and a residue of barium salt from which, on oxidation with bromine water, mucic acid was obtained. The nature of the linkage in this aldobiuronic acid was not further characterised. The methylated polysaccharide gave on methanolysis and subsequent hydrolysis: 2:3:4-trimethyl-D-xylose (30%), 2-methyl-D-xylose (23%), 2:3:4:6-tetramethyl-D-galactose (4%), and a mixture composed mainly of 3:4-dimethyl-D-xylose and methylated arabinoses in addition. Subsequent work on the seed mucilages Plantago ovata (Laidlaw and Percival, unpublished results) and Plantago lanceolata (the subject chosen for this research) has caused some doubts to be raised as to the nature of the sugars obtained from distillation fraction II - i.e., the fraction composed of a mixture of 3:4-dimethyl xylose and methylated arabinoses. Support for the presence of arabinose derivatives in this fraction was the isolation of a small quantity of

a crystalline anilide (m.p. 170°), after the removal of tetramethyl-galactose anilide, analysis revealing that this was a dimethyl-pentose anilide. Hirst, Jones and Barker[58] have since prepared a 2:4-dimethyl xylose anilide (m.p. 170°), while Plantago ovata and Plantago lanceolata have been shown to contain a good proportion of 2:4-dimethyl xylose. These results would appear to indicate that the supposed methylated arabinoses of Nelson and Percival were in fact a 2:4-dimethyl xylose. The proof for the existence of the 3:4-dimethyl xylose in this fraction also comes in for closer scrutiny in consequence of the work carried out by James and Smith[59] on gum tragacanth, and the results obtained during the present research. It is sufficient here to remark that the fraction containing the 3:4-dimethyl xylose may have consisted of a mixture of two and possibly three dimethyl xyloses, namely, 2:4, 2:3 and 3:4-dimethyl xyloses.

The mucilage obtained from Plantago lanceolata, the subject of the present research, had previously been studied by Mullan and Percival.[60] Hydrolysis of the mucilage showed the following sugars to be present: 72% Pentose (considered to be mostly D-xylose), 11% methyl pentose (not characterised) and 15% uronic acid (thought to be D-galacturonic acid, since mucic acid was obtained on oxidation with bromine water and with nitric acid). The methylated polysaccharide on methanolysis yielded four main fractions which were

shown to be (I) methyl 2:3:4-trimethyl-D-xylosides (30%), (II) methyl 3:4-dimethyl-D-xylosides (28%), (III) a mixture of the latter with methyl 2:4:6-trimethyl-D-galactosides and glycosides of lower methoxyl content (22%), and (IV) a mixture containing methyl 2:4:6-trimethyl-D-galactosides, a partly methylated methyl uronoside and unidentified glycosides. The evidence forwarded to support the existence of 3:4-dimethyl xylose in the hydrolysis products is given below.

The absence of 2:3-dimethyl xylose was shown by the failure of fraction (II) to form the crystalline anilide which this sugar readily forms.[61] Oxidation yielded a crystalline lactone (m.p. 67°), and the rate of hydrolysis was so rapid as compared with that of 2:3-dimethyl-γ-lactone that it was evidently a δ-lactone, indicating that position 4 was occupied by a methoxyl residue. The corresponding amide also in contrast to 2:3-dimethyl xylonamide, readily gave hydrazodicarbonamide on treatment with sodium hypochlorite, followed by semicarbazide.[62] That is, the dimethyl xylose must possess an α-hydroxy group, so it must be 3:4-dimethyl xylose. Confirmation of this view was obtained by oxidation with nitric acid, since after esterification an ester was obtained which was shown to be an active methyl dimethoxyglutarate of which the corresponding amide also gave a positive Weerman test. More vigorous oxidation, followed by esterification

and amide formation, yielded, in addition, a small quantity of (-)-dimethoxysuccinamide - a further proof of the existence of methoxyl residues on C_3 and C_4.

In the accompanying experimental section, using more delicate methods of separation than were available to the above workers at the period when this work was carried out, (e.g., chromatographic adsorption on an alumina column[63] and continuous extraction using two phase bi-liquid Soxhlet extractors),[64] it has been possible to isolate from this dimethyl xylose fraction crystalline 2:4-dimethyl-β-D-xylose and the crystalline amide of 2:3-dimethyl xylose, and it would appear that this fraction contains all three dimethyl xyloses - viz., 2:4, 2:3 and 3:4-dimethyl xyloses. The crystalline lactone (m.p. 67°) was shown to be identical with 2-methyl xylonolactone which was prepared from the crystalline sugar isolated from the products of hydrolysis of the methylated mucilage.

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E X P E R I M E N T A L.

PART I.

The Study of the "Free Acid" Mucilage.

Preparation of the "free acid" Mucilage.[1]

The seeds of Plantago lanceolata (500 g.) were soaked in cold water (10 ℓ.), with occasional stirring, for 36 hours. The whole mucilaginous mass was filtered through muslin and the acid polysaccharide obtained by pouring this solution slowly, with continuous stirring, into twice its volume of alcohol containing 0·7% hydrogen chloride. The acid polysaccharide was freed from chloride ions by repeated trituration with alcohol. Dehydration was completed by allowing this product to stand in ether (1 ℓ.) for 12 hours, the ether removed by filtration, and the acid polysaccharide allowed to stand in a vacuum desiccator over calcium chloride for a further 24 hours. The acid polysaccharide so obtained was fine grey-white powder (9 g., i.e. 1·8% yield). An attempt to improve the colour by boiling in alcohol (1 g. in 100 mls.) for 30 minutes was not found to be successful; however a white polysaccharide was obtained by dissolving in water and reprecipitating in acidified alcohol.

The seeds (500 g.) used above were now subjected to a further extraction with hot water (5 ℓ.) for 5 hours at 70°-80°. The solution became very dark coloured and evil smelling and very little increase in viscosity was observed. Hot extraction appeared to give only a small additional yield of polysaccharide

and was subsequently discontinued.

Cold extractions on further quantities of seed were carried out in the manner described previously, the percentage yield being found to range between 1·5 and 2. 300 g. of acid polysaccharide were thus obtained for subsequent use.

Determination of the Equivalent Weight by Titration:-[2] gave the values 1033 and 1017, the resulting mean value being 1025.

Uronic Acid Determination:-[3] gave the values 7·3%, 7·7% and 8·6%, the mean value being 7·9%.

Oxidation of the Mucilage with Sodium Periodate.[4]

(a) Estimation of formic acid liberated.

Before use in these oxidations the acid polysaccharide was carefully dried over phosphorus pentoxide. To each sample of polysaccharide taken (*ca.* 0·4 g.) water was added (120 mls.), followed by a solution of sodium periodate (25 mls. of 0·2 M.) and solid potassium chloride (5 g.). 10 ml. portions were withdrawn at various times, ethylene glycol (4 drops) added to destroy excess periodate, the mixture shaken and titrated with N/50 sodium hydroxide using methyl red as indicator. Two blank experiments were run concurrently, the first containing all the reagents, the second an aqueous solution of the acid polysaccharide. The oxidation was carried out at room temperature. NaOH = 0·0204 N

	(1) 0·4820 g.			(2) 0·3798 g.		
Time of withdrawal	Titre of 0·0204 N NaOH	No. of litres N alkali/ equivalent	No. of mols HCOOH/ equivalent	Titre 0·0204 N NaOH	No. of litres N alkali/ equivalent	No. of mols of HCOOH/ equivalent
17 hours	18·23 mls	0·7909 .	0·8	10·94 mls	0·6024 .	0·6
41 hours	34·70 mls	1·505 .	1·5	18·64 mls	1·026 .	1·0
95 hours	38·70 mls	1·679 .	1·7	30·51 mls	1·680 .	1·7
137 hours	43·76 mls	1·899 .	1·9	36·61 mls	2·016 .	2·0
400 hours	43·76 mls	1·899 .	1·9	26·61 mls	2·016 .	2·0

The above results indicate that 2 molecules of formic acid are released/equivalent of acid polysaccharide (i.e., 1025) during this oxidation, which corresponds to 2 end groups/7 sugar residues.

(b) Uptake of Periodic Acid.

In the determination of the periodic acid uptake the sample of the acid polysaccharide (*Ca.* 0·4 g.) was oxidised with sodium periodate (25 mls. of 0·2 M.). 1 ml. portions were periodically withdrawn and to each of these sodium arsenite (5 mls. of 0·1 N) was added, the reagents after mixing were allowed to stand for 15 minutes and the excess arsenite titrated against N/10 iodine, using starch (2 mls.) as indicator. The oxidation was carried out at room temperature.

I_2 = 0·1012 N .

	0·3327 g.		
Time of withdrawal	Titre of 0·1012 N iodine	Uptake of HIO_4	Uptake HIO_4 /equivalent.
Zero	-	-	-
$5\frac{1}{2}$ hours	2·03 mls.	0·01966 g.	606 g.
$23\frac{3}{4}$ hours	2·35 mls.	0·02281 g.	703 g.
77 hours	2·33 mls.	0·02257 g.	696 g.

At the maximum uptake 3·7 molecules of periodic acid were required/equivalent (1025).

Autohydrolysis of the Mucilage.

The acid polysaccharide (0·5 g.) was refluxed with water (25 mls.) on a boiling water-bath for 20 hours. A little barium carbonate was added to ensure neutralisation, the product filtered from a charred residue and the volume reduced to 10 mls. in vacuo, when the resulting solution was poured into methanol (300 mls.). The precipitate formed was removed by centrifuging and washed with methanol and ether. The alcoholic solution and washings were evaporated to dryness under reduced pressure, leaving a residue (0·087 g.) which was dissolved in water (3 mls.) and a drop of this solution was run on a paper chromatogram against the standards D-xylose, D-galactose, L-arabinose and L-rhamnose.[5] On development with ammoniacal silver nitrate spots were obtained corresponding to D-xylose and L-arabinose, but there was no trace of either D-galactose or L-rhamnose. From the relative intensity of the spots the concentration of xylose appeared to be approximately twice that of arabinose.

Oxalic acid Hydrolysis of the "free acid" Mucilage.

The mucilage (20 g.) was heated on the boiling water-bath for 20 hours with oxalic acid (300 mls. of 3%). The insoluble residue (1·1 g., i.e. 5·5%) was filtered off and the filtrate neutralised with barium carbonate in the presence of charcoal. The mixture

was kept at 80° for 15 minutes to destroy any bicarbonate present, and the filtrate evaporated to 50 mls. at 45°-50°/15 mm. This solution was poured slowly into well stirred methanol (300 mls.) to yield a barium salt which was separated by centrifuging and washed with small amounts (10 mls.) of alcohol and ether. On drying in a vacuum desiccator a yellow powder was obtained (1·60 g.). The centrifugate and washings were evaporated at 40°/15 mm. to dryness, when a hard yellow mass was obtained (15·0 g.). A portion of this hydrolysate (0·02 g.) was dissolved in water (5 mls.) and a spot run on a strip chromatogram against the standards, D-xylose, D-galactose, L-arabinose and L-rhamnose. On development a strong spot was obtained corresponding to D-xylose, with a second and much fainter spot corresponding to D-galactose. No trace of arabinose or of rhamnose could be detected.

The absence of arabinose was confirmed by the failure to obtain a crystalline diphenyl-hydrazone. The hydrolysate (0·3474 g.) and diphenylhydrazine (0·472 g.) were dissolved in a mixture of alcohol (8 mls.) and water (8 mls.), the mixture refluxed at 95° for 30 minutes and cooled to 0° for 24 hours when no sign of a precipitate of arabinose diphenylhydrazone had appeared.

The remainder of the hydrolysate was

dissolved in alcohol (20 mls.) and the solvent slowly removed in a vacuum desiccator. A white crystalline product was obtained which on washing with a little alcohol (2 mls.) and drying gave a m.p. 142° unchanged on admixture with authentic D-xylose.

DISCUSSION Of RESULTS.

When the rib grass seed (Plantago lanceolata) was soaked in cold water it gave a mucilaginous solution (cf. (6)) from which the "free acid" polysaccharide was obtained by precipitation in acidified alcohol,[7] and which gave an acid reaction to Congo Red paper. The yield of polysaccharide thus obtained was poor, values ranging between 1·5% and 2%; however, as there was very little swelling of the seeds when soaked in water it was not difficult to secure moderately concentrated aqueous solutions of the polysaccharide. (This is not found to be the case with the seed from Plantago ovata[8] when considerable swelling occurs). Subsequent hot extraction of the seeds was not found to be advantageous in recovering a further quantity of polysaccharide, and again in this instance a further comparison may be drawn with P. ovata in which the yield of hot extract polysaccharide is in excess of that obtained by cold extraction.[9]

The equivalent of the "free acid" polysaccharide was determined by titration, the mean value obtained being 1025. The uronic acid content was also determined, the three values obtained ranging from 7·3% to 8·6%, giving a mean value of 7·9%. While

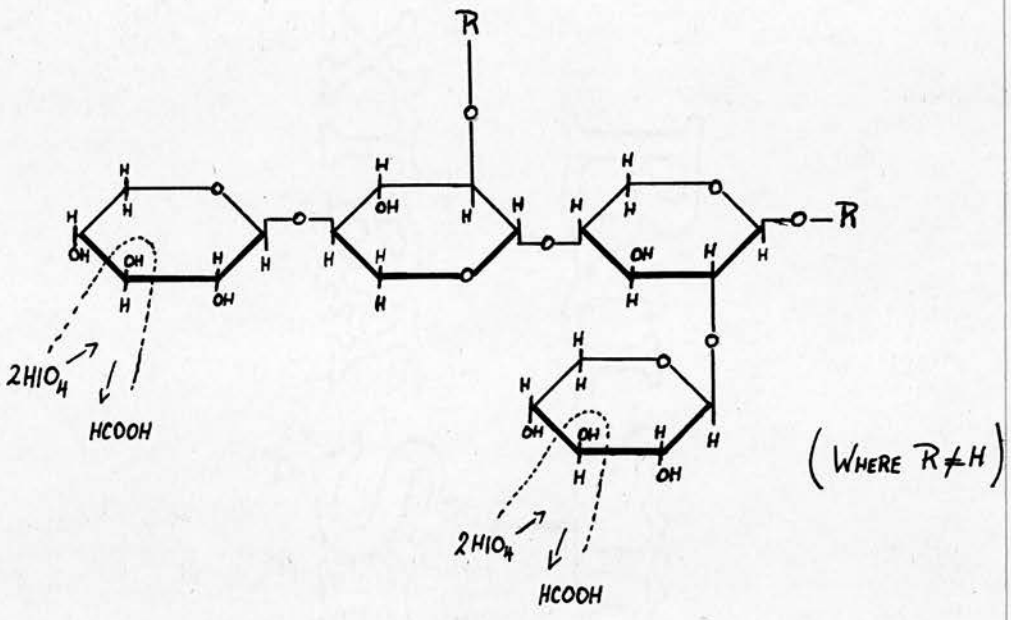
the value for the equivalent is in agreement with that obtained previously[10] (1100), the uronic acid content is not (15·2%); nor is it in agreement with the figure to be expected from a polyuronide of equivalent c. 1000 which necessitates a value c. 15% for the uronic acid content.

Autohydrolysis of the mucilage was attempted by boiling with water for 20 hours. On pouring into methanol and evaporating the alcoholic solution to dryness a syrup was obtained (17%) which was shown by the method of strip chromatography to contain D-xylose and L-arabinose. Neither galactose nor rhamnose was detected.

The polysaccharide was hydrolysed completely in 20 hours by heating with 3% oxalic acid at 100°. A black insoluble residue (5·5%) was obtained which was probably cellulosic in nature, such residues being commonly found on hydrolysis of gums and mucilages.[11] Anderson and Fireman[8] were of the opinion that it might be some essential part of the polyuronide molecule. The filtrate, after separation of this residue, was divided into two parts, namely a barium salt insoluble in alcohol and an alcohol soluble fraction consisting of a mixture of sugars, D-xylose and D-galactose being identified by the use of strip chromatography. No trace of rhamnose or of arabinose was obtained. This confirmed the failure to obtain a crystalline arabinose

diphenylhydrazone. The examination of the alcohol insoluble fraction, the barium aldobiuronate, is dealt with in Part III.

The mucilage was subjected to oxidation with sodium periodate; in the first instance the release of formic acid was investigated and in the second the total periodic acid uptake was determined. Periodic acid oxidation, first introduced by Malaprade[12] is applicable to compounds having two hydroxyl groups, or a hydroxyl and an amino group, attached to adjacent C atoms, and is characterised by the cleavage of the C-C bond. If the hydroxyl groups, or the hydroxyl and amino groups, are not attached to contiguous C atoms no oxidation takes place. This selectivity which is the outstanding characteristic of periodic acid oxidation adapts the reaction to testing for the presence of contiguous hydroxyl groups, or hydroxyl and amino groups.[13] It needs but one step for this reaction to be applied to the determination of end-groups and hence to structural problems in polysaccharide chemistry.[4] An example of such an oxidation is shown below.



It will be seen that one molecule of formic acid is released/end-group, and requires the use of two molecules of periodic acid. In the determinations carried out it was found that two molecules of formic acid were released/7 sugar residues, indicating that there were two end-groups/7 sugar residues and possibly two branches from the main chain in each repeating unit. The periodic acid uptake was lower than expected, there being only some 3·7 molecules of periodic acid used/ equivalent, and it was thought that this may have been caused by the failure of the polysaccharide to pass completely into solution in the 25 mls. of periodate used.

SUMMARY.

1. On extraction of rib grass seeds with cold water and precipitation with alcohol containing 0·7% hydrogen chloride a "free acid" polysaccharide was obtained of equivalent 1025. The percentage uronic acid was found to be 7·9. No appreciable increase in the yield of polysaccharide was obtained when extraction was continued using hot water (70°-80°).

2. Autohydrolysis of the mucilage yielded a mixture of sugars (17%) identified as D-xylose and L-arabinose of which D-xylose was present in greater quantity. Hydrolysis with oxalic acid gave an insoluble residue (5·5%), the barium salt of the aldobiuronic acid (8%) and a syrup (75%) containing D-xylose and D-galactose, the latter being present only in small quantity.

3. In the oxidation of the "free acid" mucilage with sodium periodate the formic acid released indicated the presence of two end-groups/seven sugar residues. The value obtained for the periodic acid uptake (3·7) was lower than expected and this was thought to be due to the incomplete solution of the polysaccharide in the periodate, a heterogeneous system being obtained.

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EXPERIMENTAL.

PART II.

The Study of the fully methylated "free acid" Mucilage.

Acetylation of the "free acid" mucilage.

The mucilage (150 g.) was acetylated in three 50 g. portions. Each portion of the dry mucilage was moistened with a little alcohol (10 mls.) and thoroughly dispersed in pyridine (500 mls.), when acetic anhydride (380 mls.) was added in 50 ml. portions with shaking. The mixture was then heated on a boiling water bath for 2½ hours and set aside at room temperature with occasional shaking. After 24 hours the product had become rather viscous, and a further 250 mls. of pyridine with 190 mls. of acetic anhydride were added. When a further 24 hours had elapsed, the whole mass was poured into a stream of cold water, when the acetate was precipitated as a light brown solid. The excess reagents were removed by washing the product, tied in a muslin bag, in a constant stream of water for 24 hours. The acetate was allowed to dry in air, the yield from each portion being 65 g. (i.e., 195 g. of acetate was obtained by this method).

Methylation of the Acetylated Polysaccharide.

First Methylation.

The acetate (15 g.) was stirred vigorously with acetone (300 mls.) using a mechanical stirrer. Dimethyl sulphate (160 mls.) and sodium hydroxide (400 mls. of 30%) were added simultaneously in 10 ml. and 25 ml. portions every 10 minutes. The bath

temperature was maintained initially at 40°-45°. After the addition of these reagents was completed the temperature was raised slowly to remove the acetone which was drawn off at the pump, the reaction being finally heated at 75°-80° for 1 hour. A brown solid separated along with some solid sodium sulphate, the whole was filtered hot through a hardened filter paper, the filtration being carried out as rapidly as possible as it was noticed that the solid remained hard and brittle if the filtration was rapid and the hot liquid was quickly removed. Most of the sodium sulphate was removed by washing with boiling water (1 ℓ.). Twelve 15 g. portions of acetylated polysaccharide were methylated in an identical manner.

Second Methylation.

The solid material, after washing, was returned to the flask and remethylated, the quantity of reagents used being reduced by some 25% in this second methylation: that is, the product was dissolved in acetone (225 mls.) and dimethyl sulphate (120 mls.) with 30% sodium hydroxide (300 mls.) added in 10 ml. and 25 ml. portions every 10 minutes, with continuous stirring. Filtration and washing were carried out as before.

Third Methylation.

The solid material was again returned to the flask and remethylated as above. Acetone (150 mls.)

was used for solution and dimethyl sulphate (80 mls.) with sodium hydroxide (200 mls. of 30%) added in 10 ml. and 25 ml. portions every 10 minutes with continuous stirring. After removal of the acetone as above, the bath temperature was raised to 100° for 1 hour to destroy the sodium methyl sulphate present. The resulting product was washed well with boiling water (5 ℓ.) and dried at the pump. At this stage the methylated polysaccharide appeared as a cream-coloured spongy solid. This solid was subsequently dissolved as fully as possible in chloroform (1 ℓ.), gentle refluxing being found to increase the rate of solution. This chloroform solution was then allowed to stand over anhydrous sodium sulphate for 12 hours, the chloroform then filtered off through glass wool and evaporated under reduced pressure to small volume (100 mls.), appearing at this stage as a dark-yellow viscous syrup. This syrup was poured into a large excess of light petroleum (40°-60°) (1 ℓ.) slowly with constant stirring, when a white fibrous solid was precipitated (6·0 g.). The percentage yield was found to range between 40 and 45 (of the acetylated product). Methoxyl determinations on different samples gave the values:- 34·8%, 35·0%, 34·5%, 35·6%.

$[\alpha]_D^{15°}$ (c, 0·8 in chloroform) -106·6°.

$[\alpha]_D^{15°}$ (c, 0·9 in chloroform) -104·6°.

In all, 75 g. of methylated polysaccharide were obtained.

Methylation using Thallium Ethoxide.

An attempt was made to increase the degree of methylation of the polysaccharide, fully methylated by Haworth's method using sodium hydroxide and dimethyl sulphate, by subsequent methylation by the thallium method. The methylated polysaccharide (1·17 g. OMe 35·0%) was dissolved in chloroform (50 mls.) and an equal bulk of kieselguhr added. A solution of thallium ethoxide (0·5 g.) in chloroform (10 mls.) was added, when an immediate precipitation was observed. The solvent was removed under reduced pressure and the resulting solid ground up in a mortar to a fine powder, which was refluxed with excess methyl iodide (100 g.) for 7 days, at the end of which time the mixture was found to be no longer alkaline to phenolphthalein. The methyl iodide was distilled off, the methylated polysaccharide taken up in chloroform and separated from the solid residue by centrifuging. This residue was twice washed with further quantities of chloroform (20 mls.), the chloroform solution and washings being then reduced in volume, under diminished pressure, to 5 mls. and poured into excess light petroleum (40°-60°) (400 mls.). A white fibrous solid (1·10 g.) was obtained: OMe, 34·4%. $[\alpha]_D^{15^\circ}$ (c, 1·0 in chloroform) -110°

Since the thallium method had not been successful in increasing the methoxyl content, it appeared that three methylations using sodium hydroxide

and dimethyl sulphate were sufficient for complete methylation of the polysaccharide.

Fractionation of the Fully Methylated Polysaccharide.

The methylated polysaccharide (55 g.) was dissolved in chloroform (700 mls.) by gentle refluxing for 5 hours. Because of the volumes to be handled the chloroform was divided into two equal portions, A and B.

Portion A.

To the solution of the methylated polysaccharide in chloroform (350 mls.) light petroleum (40°-60°) was added in 100 ml. portions with continuous stirring. After addition of 1500 mls. of light petroleum a stable precipitate which no longer dissolved on stirring was obtained. The precipitate was freed from the solution by decantation, and excess light petroleum added, so transforming the gelatinous mass into a crisp and more easily handled solid which on filtration and drying became white and fibrous (20 g.). On addition of a further 500 mls. (total 2 *l*.) of light petroleum to the decanted solution, a second precipitate was obtained and was separated by filtration, and on drying, this second fraction appeared as a cream-coloured granular solid (8 g.). Reduction of the volume of the filtrate to 25 mls. and further addition of light petroleum (500 mls.) gave no further precipitation.

Portion B.

Precipitation was carried out exactly as described above, and after the addition of 1400 mls. of light petroleum in 100 ml. portions with constant stirring a stable precipitate was obtained. The supernatant liquor was decanted as before and precipitation completed by addition of excess light petroleum. A white fibrous product (14 g.) was again obtained. The second fraction was a cream-coloured granular solid (13 g.) and was recovered by further addition of light petroleum (600 mls.) to the decanted solution.

That is: Initial precipitation. Final precipitation.

I A	20 g.	(73%)	II A	8 g.	(27%)
I B	14 g.	(52%)	II B	13 g.	(48%)

I A $[\alpha]_D^{17^\circ}$ (c, 1·0 in chloroform) -104·2°; OMe, 33·7%

I B $[\alpha]_D^{17^\circ}$ (c, 1·0 in chloroform) -102·2°; OMe, 33·1%.

II A $[\alpha]_D^{17^\circ}$ (c, 1·0 in chloroform) -113·9°; OMe, 36·0%.

II B $[\alpha]_D^{17^\circ}$ (c, 1·0 in chloroform) -109·1°; OMe, 33·3%.

Viscosity Determinations on the Fractionated Methylated Polysaccharide.

An Ostwald's viscometer was used, and the solvent employed was m-cresol (freshly distilled). Determinations were carried out in a thermostat (20°).

$$\eta_{sp.} = \frac{t_1 - t_2}{t_2} \qquad \text{Where } t_2 = 459.$$

c' = the concentration in g.-mols of methylated anhydroxylose residues/litre, assuming the repeating unit to be a dihydroxyanhydroxylose $C_5H_8O_4$.

TABLE of VISCOSITIES.

Substance.	Wt. in 100 mls. m-cresol (c)	Time of flow in secs. (t_1)	Temp. °C.	$\eta_{sp.}$	$\eta_{sp.}/c$	$\eta_{sp.}/c'$
m-cresol	-	459	20°	-	-	-
Fraction I A (20 g.)	0·3000 g.	1335	20°	1·908	6·362	124·7
Fraction I B (14 g.)	0·3036 g.	1363·2	20°	1·970	6·489	126·1
Fraction II A (8 g.)	0·3024 g.	935·4	20°	1·038	3·433	69·69
Fraction II B (13 g.)	0·3039 g.	951·4	20°	1·073	3·533	69·00

Hydrolysis of the Methylated Mucilage (Unfractionated).

The unfractionated methylated compound (10·0 g.) was hydrolysed by refluxing on the water bath with 3% methanolic-hydrogen chloride (250 mls.) for 18 hours. Barium chloride (1 g.) was added to precipitate any SO_4''. The cooled solution was neutralised with silver carbonate, filtered, and the filtrate, with subsequent washings, evaporated under reduced pressure to give a pale-brown non-reducing syrup (10·5 g.), any traces of moisture being removed with alcohol and benzene.

This syrup was divided into four main fractions by distillation in a high vacuum.

Fraction I. 4·63 g., b.p. 80°-100°/0·04 mm. (bath temp.),

$n_D^{10°}$ 1·4480, OMe, 54·9%.

Colourless mobile oil.

Fraction II. 1·15 g., b.p. 100°-115°/0·05 mm. (bath temp.),

$n_D^{10°}$ 1·4622, OMe, 47·1%.

Colourless, more viscous oil.

Fraction III. 1·01 g, b.p. 115°-140°/0·04 mm. (bath temp.),

$n_D^{10°}$ 1·4693, OMe, 46·9%.

Colourless, rather viscous syrup.

Fraction IV. 1·23 g., b.p. 140°-180°/0·05 mm. (bath temp.)
$\eta_D^{10°}$ 1·4762, OMe, 39·7%.

Yellow, very viscous syrup.

Residue. On attempting to raise the temperature further, the undistilled residue began to char. Distillation was discontinued.
1·12 g., OMe, 11·8%.
This still-residue was found to show a slight reducing action with Fehling's solution.

Examination of Silver Carbonate & chloride residues.

These residues obtained after the neutralisation of the methyl alcoholic hydrogen chloride used in methanolysis, were thoroughly extracted with hot water (500 mls.), the filtrate evaporated under reduced pressure to 30 mls. and poured into methanol (500 mls.). No precipitate was obtained. It had been thought possible that the silver salts of the partly methylated uronic acid, or aldobiuronic acid may have been precipitated at this stage.

Investigation of Fraction I. 4·63g., $\eta_D^{10°}$ 1·4480, OMe, 54·2%.

Refractionation.

Refractionation of fraction I was carried out using a Claisen flask with vacuum-jacketed column and

incorporated glass spiral, so increasing the efficiency of the column. Three fractions were distilled.

Fraction Ia. 1·80 g., 80°-100°/0·01 mm. (bath temp.), $\eta_D^{8°}$ 1·4447

Fraction Ib. 1·48 g., 100°-125°/0·01 mm. (bath temp.), $\eta_D^{8°}$ 1·4487.

Fraction Ic. Distilled from an ordinary flask with no fractionating column.
1·12 g., 80°-100°/0·05 mm. (bath temp.), $\eta_D^{8°}$ 1·4620, OMe, 51·6%.

Hydrolysis of Fraction Ia.

Fraction Ia (1·80 g.) was hydrolysed with nitric acid (25 mls. of 2%) for 2 hours at 100°. The resulting solution was neutralised with barium carbonate, filtered, and the residue extracted several times with boiling acetone. The filtrate and washings were evaporated under reduced pressure, any trace of moisture being removed with alcohol and benzene, leaving a viscous syrup admixed with solid barium nitrate in the flask. This mixture was extracted with dry boiling acetone (5 times 20 mls.), the acetone removed under reduced pressure to give a yellow viscous syrup (1·27 g.) which had a reducing action with Fehling's solution, and which crystallised completely on standing overnight.

On recrystallising from dry boiling ether, crystals were obtained, m.p. 84°-86°. Mixed m.p. with an authentic sample of 2:3:4-trimethyl xylose (90°-93°) was unchanged.

$[\alpha]_D^{15°}$ (c, 0·5 in water) + 19·6° (after 1 hour)

Anilide Formation.

The ether used in the above crystallisation was recovered, and on evaporation of the solvent under reduced pressure a syrup was obtained (0·190 g.). This syrup was used in anilide formation. To the syrup (0·190 g.) was added freshly distilled aniline (0·10 g.) and absolute alcohol (3 mls.). The mixture was heated under reflux at 80° for 1½ hours. The alcohol was removed in a vacuum desiccator and the resulting syrup left to crystallise. After 14 days no crystals had appeared. The absence of a crystalline anilide indicated the absence of a fully methylated galactose in this fraction (Ia).

Fraction Ib.

Fraction Ib was combined with several subsequent chromatographic fractions. (Fractions II, III and IV from chromatograph [4]). The above combination (1·644 g.) was hydrolysed with nitric acid (20 mls. of 2%) for 2 hours at 100°. Found $[\alpha]_D^{15°}$ + 29·2°. The reducing syrup recovered (1·421 g.) was allowed to crystallise as fully as possible. After three months

the crystals were removed by tiling (0·30 g.), m.p. 84°-86°. Mixed m.p. with an authentic sample of 2:3:4-trimethyl xylose (m.p. 90°-93°) was unchanged.

Anilide Formation.

The syrup remaining was converted to the anilide, as described for fraction Ia. On removal of the solvent in a vacuum desiccator partial crystallisation occurred. On recrystallisation from alcohol a white crystalline product (0·010 g.) was obtained, m.p. 190°-1°. Mixed m.p. with an authentic sample of 2:3:4:6-tetra-methyl galactose anilide (m.p. 192°) was unchanged.

Fraction Ic. 1·12 g., $n_D^{8°}$ 1·4620, OMe, 51·6%.

Since this fraction appeared to be a mixture, both from its methoxyl content and refractive index, an attempt at separation was made by chromatographic adsorption on an alumina column.(1)

In all future work the solvents were prepared as described below. Light petroleum (40°-60°) was shaken with 30% sodium hydroxide, concentrated sulphuric acid and activated alumina, filtered and distilled. Chloroform was dried over calcium chloride for 24 hours, shaken with activated alumina and distilled. Methanol was dried by refluxing over magnesium turnings (1 g./100 mls.) for 3 hours, shaken with activated alumina and distilled.

[I] Column Dimensions. 20 cms. by 1·8 cms. The column was erected using 1:1 light petroleum chloroform mixture. Fraction Ic was dissolved in this solvent mixture (25 mls.), added to the top of the column and development commenced using this solvent.

No. of Fraction.	Volume/flask	Wt. of syrup	Refractive Index.	
I	30 mls.	-	-	
II	"	0·070 g.	$n_D^{17°}$ 1·4509	Combined and Redistilled
III	"	0·130 g.	1·4508	
IV	"	0·071 g.	1·4510	
V	"	0·030 g.	1·4519	
VI	"	0·029 g.	1·4518	
VII	"	0·013 g.	-	
VIII	"	0·034 g.	1·4530	
IX	"	0·029 g.	1·4510	

Total volume of 1:1 light petroleum chloroform mixture used was approx. 300 mls.

Weight of syrup recovered using this solvent mixture was 0·406 g.

The solvent was now changed to chloroform and development continued.

X	35 mls.	0·023 g.	crystallised out on standing.	
XI	"	0·104 g.	crystallised partly $n_D^{17°}$ 1·4519.	
XII	"	0·136 g.	$n_D^{17°}$ 1·4546	Combined and Distilled
XIII	"	0·117 g.	" 1·4577	
XIV	"	0·140 g.	" 1·4571	
XV	"	0·084 g.	" 1·4533	
XVI	"	0·044 g.	" 1·4540	
XVII	"	0·035 g.	" 1·4526	
XVIII	"	0·041 g.	" 1·4500	

Total volume of chloroform used was approx. 300 mls.

Weight of syrup recovered using this solvent was 0·724 g.

Total recovery, 1·130 g. This result was thought to have been caused by traces of solvent in the fractions which were all evaporated under reduced pressure. It was thought advantageous to dry the fractions thoroughly in a high-vacuum for 10-15 minutes in the subsequent chromatographic separations. The non-reducing crystals obtained from fractions X and XI were dried on a porous tile, m.p. 75°; OMe , 43·5%. (Needles). The small quantity obtained did not permit further investigation of this glycoside.

Fractions II to VIII were combined and distilled giving a fraction Ic$_1$ 0·150 g., 80°-100°/0·02 mm. (bath temp.), $n_D^{16°}$ 1·4520, OMe, 50·4%.

Fractions XII, XIII and XIV were combined and distilled giving a fraction Ic$_2$ 0·230 g., 85°-115°/0·01 mm. (bath temp.), $n_D^{16°}$ 1·4580, OMe, 48·4%. (Calculated for $C_8H_{16}O_5$ OMe, 48·4%).

OMe on fraction XVIII $n_D^{15°}$ 1·4500 gave 42·5%. This fraction appeared to be a mixture, as indeed did Ic$_1$.

Hydrolysis of Fraction Ic$_2$.

Fraction Ic$_2$ (0·230 g.) was hydrolysed with nitric acid (6 mls. of 2%) for 5 hours at 100°. Found $[\alpha]_D^{15°}$, +25·7°. Calculated as the free dimethyl sugar +27·8°. A reducing syrup (0·154 g.) was obtained. Partial crystallisation was observed after several weeks.

These crystals were recovered by tiling (0·010 g.) and washed with light petroleum and chloroform. M.p. 103-5°. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose (m.p. 108°) was 107°-8°. The syrup (0·129 g.) was recovered from the tile by extraction with dry acetone.

Hydrolysis of Combined Fractions XV to XVIII.

This combination of fractions was hydrolysed with nitric acid as before. Found: $[\alpha]_D^{15°}$ +24·3°. A reducing syrup (0·100 g.) was obtained. No crystallisation occurred in this syrup.

Investigation of Distillation Fraction II.

100°-115°/0·05 mm., 1·15 g., $\eta_D^{10°}$ 1·4622, OMe, 47·1%

An attempt was made to purify this fraction by chromatographic adsorption on an alumina column.

[2] Column Dimensions. 35 cms. by 1·8 cms. The syrup (1·10 g.) was dissolved in 1:1 light petroleum chloroform (25 mls.) and the chromatogram developed initially using this solvent mixture.

Fractions I to VIII were either empty or contained only a trace of syrup. Total volume of light petroleum chloroform mixture used was approx. 250 mls. Development was further continued using chloroform.

No. of Fraction	Volume/ Flask.	Wt. of syrup.	Refractive Index.		
IX	35 mls.	0·012 g.	$\eta_D^{15°}$	1·4500	Combined
X	"	0·030 g.	"	1·4491	and
XI	"	0·071 g.	"	1·4520	Hydrolysed
XII	"	0·085 g.	"	1·4558	
XIII	"	0·072 g.	"	1·4557	Combined
XIV	"	0·071 g.	"	1·4570	
XV	"	0·085 g.	"	1·4596	and
XVI	"	0·070 g.	"	1·4590	
XVII	"	0·059 g.	"	1·4590	Redistilled
XVIII	"	0·041 g.	"	1·4572	
XIX	"	0·029 g.	"	1·4590	
XX	"	0·017 g.	"	1·4580	
XXI	"	0·015 g.	"	1·4562	Combined
XXII	"	0·014 g.	"	1·4527	and
XXIII	"	0·012 g.	"	1·4534	Hydrolysed

Total volume of chloroform used approx. 550 mls.

Weight of syrup recovered using this solvent was 0·683 g.

Development was continued using Methanol.

XXIV	20 mls.	0·003 g.		-	
XXV	"	0·004 g.		-	
XXVI	"	0·005 g.			
XXVII	"	0·064 g.	$\eta_D^{16°}$	1·4604	Combined
XXVIII	"	0·052 g.	"	1·4633	and
XXIX	"	0·020 g.		-	Hydrolysed

Total volume of methanol used in development was approx. 120 mls.

Weight of syrup recovered using this solvent was 0·148 g.

Total recovery : 72%.

All fractions were finally dried for 15 minutes at 40°/2 mm.

Fractions XII to XX were combined and distilled to give <u>fraction II a.</u> 84°-100°/0·01 mm. (bath temp.), 0·328 g., $\eta_D^{15°}$ 1·4598, OMe, 46·1%.

Hydrolysis of Fraction IIa.

Fraction IIa (0·320 g.) was hydrolysed with nitric acid (10 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{15°}$ + 31·9°. Calculated as the free dimethyl sugar +34·5°. A reducing syrup was obtained (0·227 g.). which partly crystallised after standing for several weeks. These crystals were recovered by tiling and washed with light petroleum and chloroform (0·015 g.), m.p. 103°-5°. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose (m.p. 108°) was 107°-8°. The syrup (0·176 g.) was recovered from the tile by extraction with dry acetone.

Hydrolysis of Combined Fractions IX, X, and XI.

These combined fractions (0·083 g.) were hydrolysed with nitric acid (4 mls. of 2%) as before.
Found $[\alpha]_D^{15°}$ + 97·5°, $\eta_D^{15°}$ 1·4701, 0·073 g.

Anilide Formation.

The anilide was formed from the hydrolysate (0·073 g.) as described previously. The solvent was removed in a vacuum desiccator, when a good yield of needle-shaped crystals was obtained (0·025 g.) which on two recrystallisations from absolute alcohol had m.p. 193°-4°. Mixed m.p. with an authentic sample of 2:3:4:6-tetramethyl galactose anilide (m.p. 192°) was 190°-2°.

Hydrolysis of Combined Fractions XXI, XXII and XXIII.

These combined fractions (0·050 g.) were hydrolysed with nitric acid as before. Found $[\alpha]_D^{15°}$ +32·0°. A reducing syrup was obtained (0·044 g.) $\eta_D^{14°}$ 1·4767.

Hydrolysis of Combined Fractions XXVII, XXVIII and XXIX.

OMe on fraction XXVII gave 35·6%. These combined fractions (0·137 g.) were hydrolysed with nitric acid as before. Found $[\alpha]_D^{15°}$ +15·3°. A reducing syrup was obtained (0·098 g.) $\eta_D^{15°}$ 1·4728.

Methylation of Fraction II by Purdie's Method.

Fraction IIa (0·083 g.), recovered from the distilling flask, was methylated by heating at 40° for 6 hours with methyl iodide (5 mls.) and silver oxide (3 g.), the silver oxide being added every 30 minutes in 0·25 g. portions with shaking. After filtration, extraction of the silver oxide with acetone and removal of the solvent in vacuo, the syrup was returned to the flask. The procedure was twice repeated. Recovery 0·065 g. The yellow oil was distilled 90-95°/0·01 mm. (bath temp.) $\eta_D^{16°}$ 1·4400, and the distillate hydrolysed with nitric acid (4 mls. of 2%) for 2 hours at 100°. A reducing syrup (0·041 g.) was obtained which crystallised completely after a few days. After two recrystallisations from dry ether, the crystalline product had m.p. 87°-90°; mixed m.p. with an authentic sample of

2:3:4-trimethyl xylose (m.p. 90°-93°) was unchanged.

The reducing dimethyl syrup extracted from the tile (p. 72) was examined in conjunction with later dimethyl xylose fractions (pp. 113-117).

Investigation of Distillation Fraction III.

1·01 g., 115°-40°/0·04 mm., $n_D^{10°}$ 1·4693, OMe, 46·9%

An attempt was made to separate this fraction into its component glycosides by chromatographic adsorption on an alumina column.

[3] Column Dimensions. 20 cms. by 1·8 cms.

Fraction III was dissolved in chloroform (15 mls.) and added to the top of the column, and development commenced using this solvent.

No. of Fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.	
I	50 mls.	0·024 g.	$n_D^{16°}$ 1·4561	Combined and Hydrolysed
II	"	0·374 g.	$n_D^{"°}$ 1·4572	
III	"	0·049 g.	n_D" 1·4580	
IV	"	0·017 g.	" 1·4571	
V	"	0·006 g.	-	
VI	"	-	-	
VII	100 mls.	-	-	

Total volume of chloroform used was approx. 400 mls.

Weight of syrup recovered using this solvent was 0·470 g.

The chromatogram was further developed using dry methanol.

VIII	50 mls.	0·257 g.	$n_D^{18°}$ 1·4679
IX	"	0·157 g.	$n_D^{19°}$ 1·4700
X	"	0·017 g.	-
XI	100 mls.	0·013 g.	-

Total volume of methanol used was approx. 250 mls. Weight of syrup recovered using this solvent was 0·444 g. Recovery: 90%.

Hydrolysis of Combined Fractions I to IV.

These combined fractions (0·455 g.) were hydrolysed with nitric acid (20 mls. of 2%) for 5 hours at 100°. A reducing syrup was obtained (0·378 g.), $[\alpha]_D^{15°}$ +44·1°, OMe, 32·7%.

Anilide Formation.

A portion of the hydrolysate (0·191 g.) was converted to the anilide as described previously. On removal of the solvent in a vacuum desiccator, complete crystallisation occurred. On recrystallisation from absolute alcohol: Crop 1, 0·041 g., m.p. 146°-53°, was again recrystallised and the 1st crop from this recrystallisation gave 0·011 g., m.p. 171-2°, OMe, 32·0% (Calculated for $C_{18}H_{23}O_5N$: OMe, 31·3%). Mixed m.p. with an authentic sample of 2:3:4-trimethyl galactose anilide gave 142°-63°. Mixed m.p. with an authentic sample of 2:4:6-trimethyl galactose anilide was unchanged.

Fraction IV was not further investigated.

Folbwing the publication[2] of a method for the quantitative separation of methylated sugars by

the preferential extraction of the fully methylated derivative using continuous extraction apparati, an attempt was made to separate the mixture of glycosides obtained on methanolysis of the fully methylated polysaccharide by this method.

Hydrolysis of the Unfractionated Methylated Mucilage.

The unfractionated methylated mucilage (10·0 g.) was hydrolysed with methyl-alcoholic hydrogen chloride (250 mls. of 3%) for 20 hours. Barium chloride (1 g.) was added to precipitate any SO_4". After neutralisation, filtration and removal of the solvent, as described previously, a pale-brown non-reducing syrup was obtained (10·9 g.).

A separation into seven main fractions was achieved by use of the Solvent Extraction method. Two solvents were used, light petroleum (b.p. 38°-40°) & chloroform, with specially constructed Soxhlet extractors which allowed intimate contact of the extracting solvent and the glycosidic solution. (Quickfit Ex. 8/25 and Ex. 10/25).

The syrup obtained by methanolysis (10·9 g.) was dissolved in water (25 mls.), and to the solution a little barium carbonate (0·5 g.) was added to ensure neutralisation. Using 200 mls. of light petroleum (38°-40°), extraction was carried out in the first instance for 3 hours. On removal of the light

petroleum under reduced pressure, a yellow oil was obtained:-

Fraction P I. 4·100 g., $n_D^{15°}$ 1·4450, 3 hours.

Using another 200 mls. of light petroleum, extraction was continued for a further 4 hours (total 7 hours), giving

Fraction P II. 0·629 g., $n_D^{15°}$ 1·4497, 7 hours.

Using 200 mls. of light petroleum, extraction was continued for a further 3 hours (total 10 hours), giving

Fraction P III. 0·314 g., $n_D^{15°}$ 1·4555, 10 hours.

The aqueous solution was now removed from the extractor, the volume of the solution and washings reduced to 25 mls. at 40°/15 mm. and added to a second Soxhlet extractor (Quickfit Ex. 10/25). the solvent changed to chloroform and extraction continued.

Using chloroform (200 mls.), extraction was initially carried out for 30 minutes. On removal of the solvent under reduced pressure, a dark-brown viscous product was obtained:-

Fraction C I. 1·380 g., $n_D^{15°}$ 1·4633, ½ hour.

Extraction was continued using chloroform (200 mls.) for a further 3 hours (total 3½ hours) giving a viscous light brown syrup -

Fraction C II. 2·919 g., $n_D^{15°}$ 1·4654, 3½ hours.

Extraction was finally carried out using chloroform (200 ml.) for a further 20 hours (total 23½ hours) giving

Fraction C III. 0·957 g., $n_D^{15°}$ 1·4738, 23½ hours, OMe, 31·2%

The aqueous solution remaining in the extractor was evaporated at 40°/15 mm. to give a very dark viscous syrup from which crystals began to separate after several weeks.

Residue. 0·498 g., $n_D^{11°}$ 1·4800.

Recovery:- 10·80g (i.e., 98%).

Refractionation of Fraction P I.

Fraction P I was dissolved in water (50 mls.) and extracted with light petroleum (38°-40°) to give three main fractions.

Extraction was carried out in the first instance with light petroleum (200 mls.) for 45 minutes. On removal of the solvent under reduced pressure, a pale yellow mobile oil was obtained which crystallised partly on standing, giving:

Fraction P Ia. 2·051 g., $n_D^{16°}$ 1·4456, 3/4 hour.

Using light petroleum (200 mls.), extraction was continued for a further 45 minutes giving

Fraction P Ib. 0·593 g., $n_D^{15°}$ 1·4431, 1½ hours.

The aqueous solution remaining was now extracted with chloroform (200 mls.) for 8 hours, giving

Fraction P Ic. 0·589 g., $n_D^{11°}$ 1·4448, 8 hours (chloroform)

On evaporation of the aqueous solution at 40°/15 mm., a residual syrup was obtained (0·090 g.).

Examination of Fraction P Ia.

The white needle-shaped crystals which appeared in this fraction were filtered and tiled, m.p. 45°-8° (0·010 g.). They were found to be insoluble in water and therefore did not appear to be glycosidic in character.

Hydrolysis of Fraction P Ia.

Fraction P Ia (1·034 g.) was hydrolysed with nitric acid (20 mls. of 2%) for 2 hours at 100°. On completion of the hydrolysis a red oil was observed on the surface, and on filtering off it cooled to a red-brown waxy material. The filtrate yielded a reducing syrup (0·802 g.) which crystallised completely, and on recrystallisation from dry ether had m.p. 87°-90°.

Anilide Formation.

The ethereal solution remaining was evaporated under reduced pressure, and the anilide prepared from the resulting syrup in the usual way. On removal of the solvent in vacuo, the anilide showed no sign of crystallising after standing for several weeks, and it was assumed a fully methylated galactose was not present in this fraction.

Hydrolysis of Fraction P Ib.

Fraction P Ib (0·590 g.) was hydrolysed as above. Found $[\alpha]_D^{15^\circ}$ +21°. A waxy, insoluble residue was also

recovered from this hydrolysis. A reducing syrup (0·465 g.) was obtained $n_D^{14°}$ 1·4589 which crystallised completely in a few days. On two recrystallisations from dry ether, had m.p. 87°-90°. The anilide was prepared as described above and did not crystallise, even after standing for several weeks.

Hydrolysis of Fraction PIc.

Fraction PIc (0·585 g.) was hydrolysed as above. Found $[\alpha]_D^{15°}$ + 39·8°. A slight waxy residue was obtained after hydrolysis, the reducing syrup obtained (0·386 g.) had $n_D^{15°}$ 1·4633 and was left to crystallise. Partial crystallisation only occurred and the crystals removed by tiling, the syrup being recovered from the tile by extraction with dry acetone which on removal under reduced pressure gave a syrup (0·360 g.) $[\alpha]_D^{15°}$ (c, 0·5 in water) + 45·2°.

Anilide Formation.

The hydrolysate (0·360 g.) was converted to the anilide as previously described, and on removal of the solvent in a vacuum desiccator a good yield of crystals was obtained, which on 3 recrystallisations from absolute alcohol had m.p. 195° (0·005 g.).

Examination of Fraction PII. 0·625 g., $n_D^{15°}$ 1·4497

Fraction P II (0·625 g.) was hydrolysed as above. (p. 79). Found $[\alpha]_D^{15°}$ +31·4°. A small amount of

waxy material was separated on completion of hydrolysis, when a reducing syrup (0·420 g.) was obtained having $n_D^{13°}$ 1·4623 $[\alpha]_D^{15°}$ (c, 0·8 in water) +39·3°.

Anilide Formation.

The anilide was prepared from the hydrolysate (0·420 g.) as described previously, and on removal of the solvent in a vacuum desiccator a good yield of crystals was obtained. Three recrystallisations from alcohol gave a product (0·012 g.) m.p. 195°. Mixed m.p. with an authentic sample of 2:3:4:6-tetramethyl galactose anilide (m.p. 192°) gave 187°-9°.

Examination of Fraction P III. 0·314 g., $n_D^{15°}$ 1·4555

This fraction was examined in conjunction with fraction C Ia (See below).

Examination of the Chloroform Fractions.

Fraction C I. 1·380 g., $n_D^{15°}$ 1·4633.

C I was separated by distillation into two portions.

Fraction C Ia. 1·092 g., $n_D^{18°}$ 1·4540, 115°/0·04 mm. (bath temp.)

OMe, 46·3%.

A solid brown residue was left in the distilling flask. Three fractions were combined:-

Fraction C Ia. 1·092 g., $n_D^{18°}$ 1·4540, OMe, 46·3%.

Fraction P III. 0·314 g., $n_D^{15°}$ 1·4555.

Fraction II. 0·320 g., $n_D^{8°}$ 1·4684 (from a

preliminary trial distillation of the hydrolysis products of the methylated mucilage).

This combination of fractions (1·726 g.) was subjected to chromatographic adsorption on an alumina column.

[4] Column Dimensions: 27 cms. by 1·8 cms.

The combination of the above fractions (1·726 g.) was dissolved in a 1:1 mixture of light petroleum chloroform (25 mls.) and added to the column. Development was commenced using this solvent.

No. of Fraction.	Volume/ Flask.	Wt. of syrup.	Refractive Index.	
I	100 mls.	-	-	
II	50 mls.	0·121 g.	$n_D^{14°}$ 1·4486	Combined
III	"	0·109 g.	$n_D^{15°}$ 1·4550	and
IV	"	0·035 g.	" 1·4583	Hydrolysed

Total volume of 1:1 light petroleum chloroform mixture used was approx. 250 mls.

Weight of syrup recovered using this solvent was 0·265 g.

Development was continued using chloroform.

V	60 mls.	0·246 g.	$n_D^{16°}$ 1·4576	
VI	"	0·394 g.	" 1·4570	Combined
VII	"	0·185 g.	" 1·4579	
VIII	"	0·120 g.	" 1·4581	and
IX	"	0·066 g.	" 1·4579	Hydrolysed.
X	"	0·036 g.	" 1·4579	
XI	"	0·015 g.	" 1·4577	
XII	"	0·011 g.	" 1·4554	

Total volume of chloroform used was approx. 500 mls.
Weight of syrup recovered using this solvent was 1·073 g.

Development was finally carried out using methanol

No. of Fraction.	Volume/ Flask.	Wt. of syrup.	Refractive Index.
XIII	50 mls.	-	-
XIV	"	0·229 g.	$n_D^{18°}$ 1·4720
XV	"	-	-
XVI	100 mls.	0·045 g.	" 1·4710

Total volume of methanol used was approx. 250 mls.
Weight of syrup recovered using this solvent was 0·274 g.
Total recovery: 94%. All fractions were dried for 15 minutes at 40°/2 mm.

Hydrolysis of the Combined Fractions V to XI.

These combined fractions (0·917 g.) were hydrolysed with nitric acid (25 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{15°}$ +32·7° (Calculated as the free dimethyl sugar +35°). A reducing syrup was obtained (0·806 g.) having $n_D^{16°}$ 1·4738; OMe, 34·6%. This fraction was seeded with a crystal of 2:4-dimethyl xylose previously obtained (p. 72) and left to crystallise as fully as possible. Crystals began to appear after 7 days. These crystals were recoverdd by tiling (0·103 g.). The crystals so obtained were washed carefully with 1:1 light petroleum chloroform mixture and with 3:1 light petroleum acetone mixture.

M.p. 109°-11°. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose (m.p. 107°-9°)[3] was unchanged. The syrupy fraction (0·680 g.) was recovered from the tile by extraction with dry acetone.

The constants found for the above crystals and accompanying syrup are recorded later (pp. 110 and 113-117)

Fractions II, III and IV were combined with distillation fraction I b and hydrolysed (See previously, p. 66).

Examination of Chloroform Fraction II. 2·919 g., $n_D^{15°}$ 1·4654

Fraction C II was further separated by fractional distillation into two portions.

C IIa. 1·764 g., $n_D^{16°}$ 1·4626, ⟨120°/0·01 mm. (bath temp.), OMe, 42·1%.

C IIb. Undistilled residue 0·847 g., $n_D^{16°}$ 1·4661, OMe, 40·7%.

Fraction C IIa was further purified by chromatographic adsorption on an alumina column.

[5] Column Dimensions: 28 cm. by 1·8 cm.

The syrup (1·764 g.) was dissolved in 1:1 light petroleum chloroform (25 mls.) and added to the column which was developed initially using this solvent mixture.

No. of Fraction.	Volume/flask	Wt. of syrup.	Refractive Index.		
I	100 mls.	-	-		
II	50 mls.	-	-		
III	"	-	-		
IV	"	-	-		
V	"	0·010 g.	Crystallised on standing.		
VI	"	0·081 g.	Crystallised partly.	$n_D^{11°}$	1·4580

Total volume of 1:1 light petroleum chloroform mixture used was approx. 350 mls.

Weight of syrup recovered using this solvent was 0·091 g.

Development was continued using chloroform.

VII	60 mls.	0·221 g.	$n_D^{11°}$	1·4580	Combined and Hydrolysed.
VIII	"	0·421 g.	"	1·4593	
IX	"	0·173 g.	"	1·4590	
X	"	0·100 g.	"	1·4591	
XI	"	0·057 g.	$n_D^{13°}$	1·4580	
XII	"	0·042 g.	"	1·4580	
XIII	"	0·031 g.	"	1·4582	
XIV	"	0·024 g.	$n_D^{16°}$	1·4566	

Total volume of chloroform used was approx. 500 mls.

Weight of syrup recovered using this solvent was 1·069 g.

Development was completed using methanol.

XV	50 mls.	0·014 g.	$n_D^{14°}$	1·4540	
XVI	"	0·349 g.	"	1·4713	
XVII	"	0·103 g.	"	1·4722	crystallised on standing.
XVIII	"	0·038 g.	"	1·4722	

Total volume of methanol used was approx. 200 mls. Weight of syrup recovered using this solvent was 0·504 g. Total recovery: 94%.

Hydrolysis of the Combined Fractions VII to XIII.

The above combination of fractions (0·912 g.) was hydrolysed with nitric acid (25 mls. of 2%) for 5 hours at 100°. Found $[\alpha]_D^{15°}$ +35·2°. (Calculated as the free dimethyl sugar +38°). A reducing syrup (0·844 g.) was obtained having $\eta_D^{16°}$ 1·4750, OMe, 35·6%. This syrup was seeded with a crystal of 2:4-dimethyl xylose and left to crystallise as fully as possible. Partial crystallisation occurred after several weeks.

The crystals were recovered by tiling and washing as described previously (0·079 g.), m.p. 109°. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose was unchanged.

The crystals were recovered from fractions XVII and XVIII by tiling and had m.p. 101°-3° $[\alpha]_D^{17°}$ (c, 0·98 in water) -56·4°. Hydrolysis with nitric acid (3 mls. of 2%) for 5 hours gave a reducing syrup (0·030 g.). A drop of a 5% solution of this syrup was run on a paper chromatogram against standards, and on development a spot appeared corresponding to 2-methyl xylose with a second faint spot corresponding to a trace of glucose.

Investigation of Fraction C IIb. 0·847 g.; $n_D^{16°}$ 1·4661; OMe, 40·7%

An attempt was made to purify this fraction by chromatographic adsorption on an alumina column.

[6] Column Dimensions. 19 cms. by 1·8 cm.

The syrup (0·847 g.) was dissolved in chloroform (25 mls.) and added to the column, development being commenced using this solvent.

No. of Fraction.	Volume/ flask.	Wt. of syrup.	Refractice Index.		
I	100 mls.	0·042 g.	$n_D^{19°}$	1·4608	Combined and Hydrolysed
II	50 mls.	0·267 g.	"	1·4587	
III	"	0·108 g.	"	1·4580	
IV	"	0·045 g.	$n_D^{20°}$	1·4581	
V	"	0·020 g.	"	1·4575	
VI	"	0·008 g.	"	1·4572	
VII	"	0·006 g.		-	

Total volume of chloroform used was approx. 400 mls.

Weight of syrup recovered using this solvent was 0·496 g.

Development was finally continued using methanol.

VIII	50 mls.	0·193 g.	$n_D^{17°}$	1·4689
IX	"	0·058 g.	$n_D^{16°}$	1·4670
X	"	0·013 g.		-
XI	100 mls.	0·023 g.		-

Total volume of methanol used was approx. 250 mls.

Weight of syrup recovered using this solvent was 0·287 g.

Recovery: 92%.

Fractions II to VI were Combined and Hydrolysed.

These combined fractions (0·432 g.) were hydrolysed with nitric acid (10 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{15°}$ +35·2°, $n_D^{18°}$ 1·4708. A reducing syrup was obtained (0·400 g.) which showed no sign of crystallising even after seeding with a crystal of 2:4-dimethyl xylose and standing for many months.

Investigation of Fraction C III. 0·957 g., $n_D^{15°}$ 1·4738, OMe, 31·2%.

Ester determination of Fraction C III.

Fraction C III (0·0824 g.) was heated with N/20 sodium hydroxide (10 mls.) for 1 hour at 100°. A blank experiment was simultaneously carried out. The remaining sodium hydroxide was back-titrated with N/20 hydrochloric acid using phenolphthalein as indicator. The difference in titre between blank and experiment was found to be 0·75 mls. of 0·04976 N HCl.
Found: $COOCH_3$, 2·6%.

Hydrolysis of Fraction C III.

A portion of this fraction (0·272 g.) was hydrolysed with nitric acid (10 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{20°}$ +26·4°. A reducing syrup was obtained (0·212 g.), $n_D^{27°}$ 1·4747, OMe, 19·9%. Crystallisation occurred after 7 days, when an attempt was made to separate the crystals by tiling, but was not successful because of the viscosity of the

accompanying syrup.

Methylation by Purdie's Method.

Fraction C III (0·502 g.) was methylated according to Purdie by heating at 40° for 6 hours with methyl iodide (25 mls.) and silver oxide (15 g.), the silver oxide being added in twelve equal portions every 30 minutes with shaking. After filtration, extraction of the silver oxide with acetone and removal of the solvent in vacuo, the syrup was returned to the flask and the procedure repeated another twice. The final product (0·447 g.) was distilled to give a colourless oil:-

0·362 g., 80°/·05 mm. (bath temp.), $n_D^{22°}$ 1·4400.

A portion of this oil (0·252 g.) was hydrolysed with nitric acid (5 mls. of 2%) for 2 hours at 100°. Found: $[\alpha]_D^{19°}$ +24·6°. A reducing syrup (0·202 g.) was recovered $n_D^{21°}$ 1·4504, OMe, 47·2%, which crystallised completely after 2 weeks and on recrystallisation from dry ether, had m.p. 87°-90°. Mixed m.p. with an authentic sample of 2:3:4-trimethyl xylose was unchanged.

A third method of separating the methylated glycosides was now attempted involving both fractional distillation and solvent extraction.

Hydrolysis of the Fractionated Methylated Mucilage.

Fractions I A and I B of the fractionated

methylated mucilage (p. 60) were combined (31·0 g.) and hydrolysed with methyl-alcoholic hydrogen chloride (600 mls. of 3%) for 22 hours. A non-reducing syrup (34·6 g.) was obtained. From previous experience it was considered advantageous to separate the fully methylated fractions by distillation. The mixture of glycosides was therefore transferred to a vacuum-jacketed Claisen flask and distilled.

Three fractions were collected:

(1) 5·217 g., 105°-120°/0·06 mm. (bath. temp.), $n_D^{18°}$ 1·4410. 49° (thermometer-well temp.)

(2) 3·252 g., 120°-5°/0·06 mm. (bath temp.) $n_D^{18°}$ 1·4414

(3) 0·610 g., 125°-30°/0·06 mm. (bath temp.), $n_D^{18°}$ 1·4418.

Recovery: 9·079 g.

It was noticed that all three fractions showed a faint blue fluorescence under the ultra-violet lamp.

The remaining glycosides were now subjected to solvent extraction, being dissolved in water (30 mls.), and after addition of barium carbonate (1 g.) the solution was extracted with light petroleum (200 mls. of 38°-40°) for 4 hours. On removal of the solvent under reduced pressure a pale-yellow oil was obtained, giving:-

Fraction P I. 4·242 g., $n_D^{19°}$ 1·4490, 4 hours.

This fraction P I was combined with the three distillation fractions (9·079 g.), and the whole redistilled from a vacuum-jacketed Claisen flask.

Again 3 fractions were obtained:

D Ia. 8·556 g., 83°-97°/0·01 mm. (bath temp.), $n_D^{17°}$ 1·4413.

D Ib. 1·579 g., 97°-110°/0·01 mm. (bath temp.), $n_D^{19°}$ 1·4420.

D Ic. 1·371 g., residual remaining in flask, $n_D^{17°}$ 1·4542, OMe, 50·1%.

Extraction of the main bulk was continued using light petroleum (200 mls.) for a further 20 hours (total 24 hours), giving a yellow syrup.

Fraction P II. 3·028 g., $n_D^{15°}$ 1·4560, OMe, 47·3%, 24 hours.

Extraction was continued using light petroleum (200 mls.) for a further 70 hours (total 94 hours), giving a yellow syrup.

Fraction P III. 7·654 g., $n_D^{18°}$ 1·4595, OMe, 47·0%, 94 hours.

The aqueous solution was removed from the Soxhlet extractor (Ex. 8/25), the volume of the solution and washings reduced at 40°/15 mm. to 30 mls., added to the second extractor (Ex. 10/25) and extraction continued

using chloroform (200 mls.) for 3/4 hour, when on removal of the solvent under reduced pressure a deep brown coloured product was obtained.

Fraction C I. 1·915 g., $n_D^{17°}$ 1·4690, OMe, 37·5%, 3/4 hour.

Extraction was continued using chloroform (200 mls.), and when after 1¼ hours a marked lightening of the aqueous solution was observed, extraction was stopped. On removal of the solvent a dark-brown product was obtained.

Fraction C II. 0·560 g., $n_D^{18°}$ 1·4590, 2 hours.

Fractions C I and C II were combined (2·475 g.).

Extraction was now finally continued using chloroform (200 mls.) for a further 22 hours (total 24 hours), giving a pale-brown viscous syrup.

Fraction C III. 4·678 g., $n_D^{19°}$ 1·4746, OMe, 29·0%, 24 hours.

On evaporation of the aqueous solution at 40°/15 mm. a dark brown glass was obtained.

Residue: 2·699 g.

Recovery: 97·8%.

Examination of Fractions D Ia and D Ib.

These two distillation fractions were combined (10·135 g.) and hydrolysed with nitric acid (150 mls.

of 2%) for 5 hours at 100°.

$[\alpha]_D^{17°}$ Initial +18·8°, 1 hour +24·5°, 2 hours +18·2°
3 hours +15·7°, 4 hours +15·0°, 5 hours +15·0°.

A reducing syrup (8·829 g.) was obtained $n_D^{15°}$ 1·4600 which crystallised almost completely overnight. The crystals were carefully washed with dry ether, the washings evaporated in vacuo and the resulting syrup allowed to crystallise out once more. This process was repeated until a syrup was obtained (0·303 g.) which could not be induced to crystallise further. This syrup was passed through an alumina column (28 cm. by 1·8 cm.) using a mixture of 5 chloroform 1 methanol for development.

No. of Fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.
I to IX	50 mls.	-	-
X	"	0·112 g.	$n_D^{18°}$ 1·4560
XI	"	0·045 g.	" 1·4568
XII	"	0·005 g.	" 1·4550
XIII	175 mls.	0·004 g.	-

Total volume of 5:1 chloroform methanol used was approx. 800 mls.

Weight of syrup recovered using this solvent was 0·166 g.

Fractions X to XIII were combined and had $[\alpha]_D^{17°}$ (c, 1·09 in water) +3·7°.

A small portion of the solution used in the

rotation was run on a strip chromatogram[4] against standards. However, on development, only one spot was obtained which appeared to correspond to 2:3:4-trimethyl xylose (R.G. value 0·94), but 2:3:5-trimethyl arabinose (R.G. value 0·95) may also have been present.

The remainder of the solution was used in the preparation of the lactone. To this solution (5 mls.) liquid bromine (1 ml.) was added and the reactants allowed to stand at room temperature for 6 days, when on the removal of the bromine by aeration, the reducing action was found to have disappeared. The solution was neutralised with silver carbonate and the Ag^+ removed with hydrogen sulphide. The excess of hydrogen sulphide was in turn removed by aeration, and the solution concentrated to a syrup which was heated at 100°/ 15 mm. for 3 hours to effect lactonisation. This product was distilled to give a pale yellow syrup:

0·127 g., 100°-110°/0·01 mm. (bath temp.),

$n_D^{17°}$ 1·4585.

Partial crystallisation occurred on standing, the crystals separated by tiling (0·012 g.) had m.p. 47°-8°. Mixed m.p. with 2:3:4-trimethyl xylonolactone (m.p.47°-8°) was unchanged.

The tile was extracted with dry boiling acetone, when a yellow syrup (0·0679 g.) was recovered.

$[\alpha]_D^{18°}$ (c, 0·679 in water).

Initial +6·4, 3 hours ±0°, 15 hours +3·2°,

40 hours +9·5°, 64 hours +12·7°, constant value. It did not appear therefore that 2:3:5-trimethyl-δ-arabonolactone[5] was present to any extent in the fully methylated fractions.

The crystalline 2:3:4-trimethyl xylose recovered from this fraction on two recrystallisations from dry ether had m.p. 88°-90°.

$[\alpha]_D^{21°}$ (c, 1·2 in water):

Initial +53·8°, 15 minutes +35·3°, 30 minutes +26·9°, 45 minutes +23·5°, 1 hour +20·2°, constant value.

It would appear that the sugar was present in the α form.

Formation of the Lactone from 2:3:4-trimethyl xylose.

Crystalline 2:3:4-trimethyl xylose (0·800 g.) was dissolved in water (10 mls.) and liquid bromine (1·2 mls.) added. The reactants were kept at room temperature for 5 days, when on removal of the bromine by aeration the reducing action was found to have disappeared. Lactone formation was carried out as described previously. On distillation: 0·650 g., 110°-125°/0·04 mm. (bath temp.) the distillate crystallised immediately, m.p. 46°.

Two recrystallisations from dry ether gave fine white needles, m.p. 53°. (Found: C, 50·6 ; H, 7·42; OMe, 51·1. $C_8H_{14}O_5$ requires C, 50·5; H, 7·4; OMe, 48·9%).

Rotation of 2:3:4-trimethyl-δ-xylonolactone. cf.(6)

$[\alpha]_D^{12°}$ (c, 1·60 in water).

Initial +1·3°, 3 hours +2·0°, 10·5 hours +4·0°, 23 hours +6·6°, 47·5 hours +10·5°, 71·5 hours +14·5°, 95·5 hours +16·5°, 120 hours +19·1°, constant value.

Preparation of 2:3:4-trimethyl xylose anilide.

Crystalline 2:3:4-trimethyl xylose (0·480 g.) was converted to the anilide in the usual way. On removal of the solvent in a vacuum desiccator a syrup was obtained which was seeded with a crystal of authentic 2:3:4-trimethyl xylose anilide, and left to crystallise.

The partially crystalline product was tiled, giving:

0·160 g. m.p. 98°-99°. Two recrystallisations from ether raised the m.p. to 100°-101°. (Found: C, 61·4; H, 7·64; N, 5·32; OMe, 35·9. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2; OMe, 34·8%).

Examination of Fraction D Ic.

An attempt was made to purify this fraction by chromatographic adsorption on an alumina column. A number of similar fractions were combined with D Ic, all being chromatographed together.

Fraction D Ic. 1·371 g., $n_D^{17°}$ 1·4542, OMe, 50·1%

Fractions { I. 0·807 g., $n_D^{19°}$ 1·4478.

II. 0·690 g., " 1·4548.

These were obtained from the chromatographic adsorption [8] of P II (see later, p. 103).

Fraction I. 0·460 g. $n_D^{9°}$ 1·4605.

A preliminary trial distillation fractionation 80°-100°/0·04 mm. (not recorded).

That is, total amount to be separated was now 3·328 g.

[7] Column Dimensions: 23 cm. by 3·5 cm.

The mixture of glycosides (3·328 g.) was dissolved in 25 mls. of a 4:1 mixture of light petroleum chloroform, added to the column and development commenced using this solvent mixture.

No. of fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.	
I	100 mls.	-	-	
II	50 mls.	-	-	
III	"	0·086 g.	$n_D^{17°}$ 1·4502	Crystallised partly on standing.
IV	"	0·077 g.	" 1·4489	Combined with VI to XI and Hydrolysed.
V	"	0·137 g.	" 1·4482	

Total volume of 4:1 light petroleum chloroform used was approx. 300 mls.

Weight of syrup recovered using this solvent was 0·300 g.

Development was continued using 2:1 light petroleum chloroform.

No. of fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.		
VI	50 mls.	0·101 g.	$n_D^{18°}$	1·4482	Combined with IV and V and Hydrolysed.
VII	"	0·115 g.	"	1·4480	
VIII	"	0·163 g.	"	1·4482	
IX	"	0·216 g.	$n_D^{19°}$	1·4483	
X	"	0·186 g.	"	1·4482	

Total volume of 2:1 light petroleum chloroform used was approx. 250 mls.

Weight of syrup recovered using this solvent was 0·781 g.

Development was continued using chloroform.

XI	70 mls.	0·139g.	$n_D^{19°}$	1·4486	
XII	"	0·129 g.	"	1·4520	Yellow-coloured fractions.
XIII	35 mls.	0·163 g.	"	1·4542	Combined and Hydrolysed.
XIV	"	0·214 g.	"	1·4547	
XV	70 mls.	0·388 g.	$n_D^{20°}$	1·4550	Combined and Hydrolysed.
XVI	"	0·348 g.	"	1·4550	
XVII	140 mls.	0·535 g.	$n_D^{17°}$	1·4560	

Total volume of chloroform used was approx. 500 mls.

Weight of syrup recovered using this solvent was 1·816 g.

Development was continued using methanol.

XVIII	50 mls.	0·102 g.	$n_D^{17°}$	1·4555
XIX	75 mls.	0·242 g.	$n_D^{15°}$	1·4568
XX	"	-		-

Total volume of methanol used was approx. 200 mls. Weight of syrup recovered using this solvent was 0·344 g. Overall recovery; 88%.

Fractions IV to XI were Combined and Hydrolysed.

These combined fractions (1·036 g.) were hydrolysed with nitric acid (25 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{16°}$ +40·6°. A reducing syrup (0·884 g.) was obtained having $n_D^{18°}$ 1·4585, OMe, 51·1%. This product was seeded with a crystal of 2:3:4-trimethyl xylose, and left to crystallise. However, after several weeks no crystallisation had occurred and the syrup was converted into the anilide in the usual way. It was noticed that on allowing the alcoholic solution of aniline to stand in contact with the syrup at room temperature, partial anilide formation had occurred after 12 hours. In this way 0·189 g. were recovered, m.p. 188°. After heating the solution in the conventional way, a further two crops were obtained. 0·281 g. m.p. 188°-9°, and 0·068 g. m.p. 184°-6°. On two recrystallisations from absolute alcohol the m.p. was raised to 192-3°. Mixed m.p. with an authentic sample of 2:3:4:6-tetramethyl galactose anilide (m.p. 192°) was unchanged.

Fractions XII, XIII and XIV were Combined and Hydrolysed.

This combination of fractions (0·357 g.) was

hydrolysed with nitric acid (15 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{17°}$ +40·3°. A reducing syrup was obtained (0·306 g.) having $\eta_D^{25°}$ 1·4688, OMe, 40·9%. This syrup was seeded with a crystal of 2:4-dimethyl xylose and left to crystallise. No crystallisation occurred, even after several weeks, and the anilide was prepared as previously described. On cooling, a crop of white needle-shaped crystals separated, 0·112 g., m.p. 188°-9°, and on removal of the solvent in a vacuum desiccator a second crop was obtained: 0·056 g., m.p. 183°-6°. On two recrystallisations from absolute alcohol, the m.p. was 192-3°.

$[\alpha]_D^{19°}$ (c, 0·55 in acetone) -74·9°. OMe, 37·1%.
(Calculated for $C_{16}H_{25}O_5N$ OMe, 39·8%).

In all, 0·700 g. of 2:3:4:6-tetramethyl galactose anilide was recovered from these two fractions.

Fractions XV to XVIII were Combined and Hydrolysed.

These combined fractions (1·264 g.) were hydrolysed with nitric acid (30 mls. of 2%) for 5 hours at 100°. Found $[\alpha]_D^{17°}$ +37·6°. A reducing syrup (1·110 g.) was obtained having $\eta_D^{19°}$ 1·4722, OMe, 31·7%. This fraction was seeded with a crystal of 2:4-dimethyl xylose and placed on a high-vacuum pump for 12 hours at 30°/0·5 mm., when crystallisation spread throughout the syrup. The crude product was separated by tiling (0·701 g.). This crude product was dissolved in the

minimum quantity of dry acetone, solution being effected by warming, and to the warm solution dry, light petroleum (80°-100°) was carefully added. Crystallisation occurred initially at the interface of the two liquids and gradually spread throughout the solution. 0·330 g. of brittle white needles were obtained, m.p. 108°-10°. (Found: C, 46·8; H, 8·0; OMe, 33·8. $C_7H_{14}O_8$ requires C, 47·1; H, 7·9; OMe, 34·8%).

$[\alpha]_D^{19°}$ (c, 2·33 in water). cf. (3).

17 minutes +3·8°, 37 minutes +15·8°,

67 minutes +20·9°, 2 hours +22·7°, 3 hours +22·7°,

24 hours +22·7°, constant value.

From these rotational changes it would appear that the sugar was present in the β form.

The syrupy fraction (p. 100) was recovered from the tile by extracting with acetone.

Preparation of the Osazone from the syrupy Dimethyl fraction.

The syrup used was that recovered after tiling the above crystals. This syrupy fraction (0·176 g.) was dissolved in water (5 mls.) containing glacial acetic acid (1 ml.). Phenylhydrazine (0·28 g.) and a little sodium bisulphite were added, and the mixture heated on a water bath at 90° for 1 hour. On cooling, a dark red-brown tar separated out, the liquid decanted off and the heating continued for a further

3 hours. The tar which separated out on cooling was likewise removed. The tar was dissolved in ether, shaken with a solution of dilute (2 N) hydrochloric acid and then with N/10 sodium bicarbonate solution. The ethereal solution was dried over calcium chloride and the volume of the solution reduced to 10 mls. in vacuo, when it was poured into excess light petroleum (200 mls. of 40°-60°), a brown solid separating out (0·005 g.). This product was carefully dried over phosphorus pentoxide and had OMe, 6·6%, On complete evaporation of the light petroleum at 20°/15 mm. a red viscous tar was obtained which, after drying over phosphorus pentoxide, had OMe, 8·0%. ($C_{18}H_{22}O_3N_4$ requires OMe, 9·3%.). It would appear that in the formation of the osazone a methoxyl grouping was being removed from position C_2 giving a value for the percentage OMe corresponding to that for the osazone of a monomethyl pentose.

Examination of Fraction P II. 3·028 g., $\eta_D^{15°}$ 1·4560, OMe, 47·3%.

An attempt was made to purify this fraction by chromatographic adsorption on an alumina column. Two similar fractions were combined with P II.

Fraction P II. 3·028 g., $n_D^{15°}$ 1·4560, OMe, 47·3%

Fractions { II 0·281 g., $n_D^{18°}$ 1·4588. From chromatographic adsorption [9] of fraction P III (p.104)
XVII 0·425 g., $n_D^{19°}$ 1·4594

Total: 3·734 g.

[8] Column Dimensions. 30 cm. by 1·8 cm.

These 3 fractions (3·734 g.) were dissolved in 2:1 light petroleum chloroform mixture (25 mls.) and added to the column which was then developed using this mixture of solvents.

No. of Fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.	
I	100 mls.	0·807 g.	$n_D^{19°}$ 1·4478	Combined with [7]
II	50 mls.	0·690 g.	" 1·4548	(see previously, p. 97)
III	"	0·177 g.	" 1·4560	Combined and
IV	"	0·224 g.	" 1·4550	
V	"	0·354 g.	" 1·4552	
VI	"	0·324 g.	" 1·4552	

Total volume of 2:1 light petroleum chloroform used was approx. 350 mls.

Weight of syrup recovered using this solvent was 2·576 g.

Development was continued using chloroform.

VII	70 mls.	0·446 g.	$n_D^{20°}$ 1·4559	Hydrolysed.
VIII	"	0·529 g.	" 1·4555	
IX	"	0·082 g.	" 1·4565	
X	"	0·005 g.	-	
XI	"	-	-	

Total volume of chloroform used was approx. 350 mls.
Weight of syrup recovered using this solvent was 1·062 g.
Development was carried out finally using methanol.

XII 100 mls. 0·025 g. $n_D^{19°}$ 1·4690.

Overall recovery: 98%.

All fractions were dried at 30°/2 mm. for 15 minutes.

Fractions III to IX were Combined and Hydrolysed.

This combination of fractions (2·093 g.) was hydrolysed with nitric acid (50 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{19°}$ +29·3°. A reducing syrup was obtained (1·895 g.) having $n_D^{22°}$ 1·4717, OMe, 31·3%. The syrup was seeded with a crystal of 2:4-dimethyl xylose, and kept at 30°/0·5 mm. for 10 hours, when crystallisation had spread throughout the fraction. The crude product separated by tiling (0·690 g.) was recrystallised as described previously (p.101) giving 0·135 g., m.p. 108°-10°. The syrup was recovered from the tile by extraction with acetone.

Examination of Fraction P III. 7·654 g., $n_D^{18°}$ 1·4595, OMe, 47·0%.

This fraction was purified by chromatographic adsorption on an alumina column.

[9] Column Dimensions. 20 cm. by 3·5 cm.

Fraction P III (7·654 g.) was dissolved in a

1:1 light petroleum chloroform mixture (25 mls.) and added to the column which was developed using this solvent.

No. of fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.		
I	100 mls.	-	-		
II	65 mls.	0·281 g.	$n_D^{18°}$	1·4588	Combined with [8] (see previously, p. 103)
III	"	1·805 g.	"	1·4572	Combined and Hydrolysed
IV	"	0·545 g.	"	1·4582	
V	"	0·337 g.	"	1·4569	
VI	"	0·277 g.	"	1·4581	
VII	"	0·242 g.	"	1·4578	
VIII	"	0·663 g.	$n_D^{20°}$	1·4571	
IX	"	0·402 g.	"	1·4570	
X	"	0·370 g.	"	1·4570	

Total volume of 1:1 light petroleum chloroform used was approx. 700 mls.

Weight of syrup recovered using this solvent mixture was 4·922 g.

Development was continued using chloroform.

XI	60 mls.	0·356 g.	$n_D^{20°}$	1·4559	Combined and Hydrolysed.
XII	"	0·346 g.	"	1·4552	
XIII	"	0·357 g.	"	1·4563	
XIV	"	0·270 g.	$n_D^{21°}$	1·4556	
XV	"	0·192 g.	$n_D^{18°}$	1·4558	
XVI	70 mls.	0·141 g.	$n_D^{19°}$	1·4567	

Total volume of chloroform used was approx. 400 mls.

Weight of syrup recovered using this solvent was 1·662 g.

Development was continued using methanol.

No. of fraction.	Volume/ flask.	Wt. of syrup.	Refractive Index.		
XVII	70 mls.	0·425 g.	$n_D^{19°}$	1·4594	Combined with chromatograph [8] (see previously, p.103)
XVIII	"	0·185 g.	"	1·4674	Combined.
XIX	140 mls.	0·076 g.	$n_D^{20°}$	1·4672	
XX	"	0·044 g.		-	Crystallised.

Total volume of methanol used was approx. 450 mls.

Weight of syrup recovered using this solvent was 0·730 g.

Overall recovery: 7·314 g. (96%).

(All fractions were dried for 15 minutes at 40°/2 mm.).

Fractions III to X were Combined and Hydrolysed. (P IIIa)

These combined fractions (3·917 g.) were hydrolysed with nitric acid (80 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{19°}$ +40·1°. A reducing syrup (3·564 g.) was obtained having $n_D^{19°}$ 1·4743, OMe, 35·8%. This fraction was seeded with a crystal of 2:4-dimethyl xylose and placed on a high vacuum pump for 12 hours at 20°/0·5 mm., when crystallisation spread throughout the fraction. The crude product was separated by tiling (1·100 g.) and recrystallised from acetone by addition of light petroleum (80°-100°) as described previously (p. 101) giving 0·409 g., m.p. 108°-10°.

The syrup was recovered from the tile by extraction with acetone.

Fractions XI to XVI were Combined and Hydrolysed. (P IIIb)

These combined fractions (1·492 g.) were hydrolysed with nitric acid, as above. Found: $[\alpha]_D^{19°}$ +25·5°. A reducing syrup (1·365 g.) was obtained having $\eta_D^{20°}$ 1·4715, OMe, 33·3%. This fraction was seeded and thoroughly dried as described above but no crystallisation occurred.

Lactone Formation.

The syrup (1·239 g.) was dissolved in water $[\alpha]_D^{15°}$ (c, 0·735 in water) +27·2°, and liquid bromine (1·5 mls.) added. The reactants were left at room temperature for 5 days, when on removal of the bromine by aeration the reducing action was found to have disappeared. Lactone formation was carried out as described previously (p. 94). Two fractions were collected on distillation.

(I) 0·673 g., $\eta_D^{17°}$ 1·4660, 125°-35°/0·05 mm. (bath temp.), OMe, 32·3%.

(II) 0·234 g., $\eta_D^{17°}$ 1·4670, 135-50°/0·03 mm. (bath temp.)

Fraction (II) was seeded with a crystal of supposed 3:4-dimethyl xylonolactone,[7] and left to crystallise. No crystallisation had occurred after 12 months.

Titration of the Lactone. (fraction (I)) with N/20 Sodium Hydroxide.

The lactone (0·0284 g.) was dissolved in water (5 mls.), 2 drops of phenolphthalein added and titrated immediately with sodium hydroxide (0·05279 N). Initially the colour faded quite rapidly in the cold, but the rate of hydrolysis gradually decreased until gentle warming was required near

the end point. Titre of 0·05279 N sodium hydroxide required was 3·05 mls. (Calculated titre for $C_7H_{12}O_5$ was 3·06 mls.)

Rotation of Lactone.

$[\alpha]_D^{15°}$ (c, 0·809 in water). cf. (8)

Initial +63·1°, 1 hour +63·1°, 3 hours +63·1°, 6 hours +63·
24 hours +65·5°, 48 hours +65·5°, 72 hours +65·5°, 96 hours
+64·3°, 192 hours +60·6°, 264 hours +56·9°, 336 hours +56·6
384 hours +54·4°, 576 hours +54·4°

From the rotational change this appeared to be a γ-lactone.

Preparation of Amide.

The lactone (0·292 g.) was treated with dry concentrated methyl alcoholic ammonia (8 mls.) at 0° for 3 days. The solvent was removed in a vacuum desiccator, when a solid crystalline mass separated. On two recrystallisations from alcohol it had m.p. 132°-3°, 0·148 g.

$[\alpha]_D^{16°}$ (c, 1·3 in water) +49·7°. cf. (9)

Mixed m.p. with authentic 2:3-dimethyl xylonamide (m.p. 133°) was unchanged.

(Found: C, 43·5 ; H, 7·9 ; N, 7·0 ; OMe, 31·3.
$C_7H_{15}O_5N$ requires C, 43·5 ; H, 7·7 ; N, 7·25 ;
OMe, 32·1%).

Weerman Test.

A Weerman test was carried out on the crystalline amide, and simultaneously a control was run with gluconamide. The weight of crystalline amide taken was

0·0240 g., and weight of gluconamide 0·0223 g. Both samples were dissolved in water (0·1 ml.), sodium hypochlorite solution (0·15 ml.) added, and the mixture kept at 0° for 3 hours. The excess hypochlorite was destroyed by addition of a concentrated solution of sodium thiosulphate (4 drops), then sufficient solid anhydrous sodium acetate was added to saturate the solution, filtered, and washed with a saturated solution of semicarbazide hydrochloride (1·5 mls.). After standing for 12 hours at 0° to allow complete precipitation, the control gave 0·0055 g. of hydrazodicarbonamide (40·8% of theoretical), while the test solution showed no sign of a precipitate: that is, a negative Weerman confirming the presence of a substituent on C_2.

Attempted Phenylhydrazide formation.

The lactone (0·055 g.) was dissolved in alcohol and freshly distilled phenylhydrazine (0·051 g.) added. The mixture was refluxed at 80° (bath. temp.) for 1 hour. No crystalline derivative could be obtained on removal of the solvent.

Oxidation with Periodic Acid.[10]

A portion of the amide (m.p. 132°-3°, 0·0274 g.) was dissolved in water (2 mls.) and sodium bicarbonate (2 mls. of N) added, followed by periodic acid (2 mls. of 0·3 M.). The solution was mixed and allowed to

stand at room temperature for 1 hour, when hydrochloric acid (3 mls. of N) and sodium arsenite (2 mls. of N) were added with mixing. When the precipitate and yellow colour had completely disappeared sodium acetate (2 mls. of N) and dimedon reagent (1 ml. of solution containing 85 mg./ml. of 95% alcoholic solution) were added. A precipitate appeared immediately and was allowed to reach completion by standing at room temperature for 12 hours, filtered, washed with water and dried at 95° for 1 hour. Weight of dimedon complex precipitated was 0·0402 g., giving the weight of formaldehyde as (0·0402 × 0·1027)g.
Calculated for $C_7H_{12}O_6N$, the percentage theoretical yield was 97.

This oxidation confirms the absence of methoxyl groups on C_4 and C_5. An authentic sample of 2:3-dimethyl xylonamide gave a percentage yield of 94 on oxidation with periodic acid and precipitation of the dimedon complex as described above.

Investigation of the Crystalline sugar, m.p. 108°-10°.

Oxidation with Periodic Acid.

A portion of the crystalline sugar (0·0339 g.) was oxidised with periodic acid, and the formaldehyde estimated as the dimedon complex. No precipitate was obtained, indicating that C_4 was occupied.

Lactone Formation.

The crystalline sugar (0·132 g.) was dissolved in water (5 mls.), and liquid bromine (1·5 mls.) added. The reactants were kept at room temperature for 5 days, and then at 50° for 30 hours. On removal of the bromine by aeration, the reducing action was found to have disappeared. Lactone formation was continued as previously described.

Distillation gave: 0·0645 g., 130°-5°/0·01 mm. (bath. temp.), last drop $n_D^{14°}$ 1·4800.

$[\alpha]_D^{15°}$ (c, 0·645 in water).

Initial -4·7°, 2 hours -1·6°, 5½ hours +4·7°, 24 hours +17·1°, 48 hours +26·4°, 72 hours +26·4° (constant value).

From the rotational change this appeared to be a δ-lactone.

Titration with Sodium Hydroxide.

2 mls. of the above solution of the lactone (0·0129 g.) was titrated with sodium hydroxide (0·05165 N) using phenolphthalein as indicator. The volume of N/20 sodium hydroxide required was 1·48 mls. (Calculated for $C_7H_{12}O_5$ 1·42 mls.). Gentle warming was required towards the end point.

Preparation of the Amide.

The syrupy lactone (0·054 g.) was treated with dry methyl-alcoholic ammonia (2 mls.) at 0° for 2 days.

On removal of the solvent in a vacuum desiccater the syrupy amide was obtained (0·055 g.).

Weerman Tests.

A Weerman test was carried out on the syrupy amide, and simultaneously a control was run with gluconamide. The weight of syrupy amide taken was 0·0548 g., and weight of gluconamide taken was 0·0645 g. Both samples were dissolved in water (0·5 mls.), sodium hypochlorite solution (0·7 mls.) added, and the mixture kept at 0° for 3 hours. The excess hypochlorite was destroyed by addition of a concentrated solution of sodium thiosulphate (6 drops), then sufficient solid anhydrous sodium acetate was added to saturate the solution, filtered and washed with a saturated solution of semicarbazide hydrochloride (3 mls.). After standing for 12 hours at 0° to allow complete precipitation, the control gave 0·0190 g., m.p. 254° (50% theoretical), while the test solution showed no sign of a precipitate: that is, a negative Weerman confirming the presence of a substituent on C_3.

Anilide Formation.

The anilide was prepared from the crystalline 2:4-dimethyl xylose (0·051 g.) as previously described (p. 66). On one recrystallisation from alcohol, m.p. was 159°-61°. On subsequent recrystallisations the m.p. dropped. Second recrystallisation: m.p. 153°.

Third: m.p. 133°. (Quot. m.p. 170°[3]). Decomposition appeared to be occurring on recrystallisation. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose anilide, however, showed no depression.

Investigation of the Syrupy dimethyl pentose fraction.

This fraction was that recovered from the tile by extraction with acetone after the removal of the crystalline 2:4-dimethyl xylose (3·232 g.).

Osazone formation. (see p. 101).

Periodic Acid Oxidation.

A quantitative estimation of the formaldehyde liberated by periodic acid oxidation was carried out on this syrupy fraction, the method being as described previously. Assuming the syrup to consist only of dimethyl pentoses, then from the weight of dimedon complex precipitated, the percentage of the sugar not substituted on C_4 and C_5 was found to be 9·0 and 9·6, giving a mean value of 9·3%.

Strip Chromatography.[4]

A drop of a 5% aqueous solution of this syrupy fraction was run on a strip chromatogram against standards. On development, two spots appeared corresponding to 2:4-dimethyl xylose (R.G. value 0·66) and 2:3-dimethyl xylose (R.G. value 0·74), while the relative intensity of the spots suggested a 40:60

mixture of the two sugars.

Lactone Formation.

The syrup (0·783 g.) was dissolved in water (10 mls.) and bromine (2 mls.) added. After 5 days at room temperature, oxidation was completed by heating at 50° for 4 hours. On removal of the bromine by aeration the solution was found to be no longer reducing when tested with Fehling's solution. Lactonisation was carried out as previously described.

On distillation a yellow syrup was obtained, 0·562 g., 135-40°/0·05 mm. (bath temp.), $n_D^{15°}$ 1·4750. $[\alpha]_D^{15°}$ (c, 1·36 in water).

Initial +65·5°, 1 hour +63·3°, 5 hours +52·3°, 17½ hours +50·1°, 24½ hours +51·5°, 43 hours +55·2°, 113 hours +54·5°, 162 hours +51·5°, 210 hours +51·5°, 282 hours +50·1°, 378 hours +49·3°, 600 hours +48·6°.

Titration of the mixed Lactones.

The mixture of lactones (0·0398 g.) was dissolved in water (5 mls.) and titrated with N/20 sodium hydroxide using phenolphthalein as indicator. The volume of 0·0509 N sodium hydroxide required was 4·05 mls. (Calculated for $C_7H_{12}O_6$ 4·44 mls.). This indicated the presence of some 10% free sugar.

Amide Formation.

The syrupy lactone (0·080 g.) was dissolved in

methyl-alcoholic ammonia (2 mls.) and the reaction kept at 0° for 3 days, when on removal of the solvent a syrupy amide was obtained. $n_D^{17°}$ 1·4735.

Weerman Test.

A Weerman test was carried out on the syrupy amide and on a control of gluconamide as described previously (p.108). The weight of syrupy amide taken was 0·0630 g. and of gluconamide 0·0642 g. The control yielded 0·0165 g. of hydrazodicarbonamide (42·5% theoretical), while the syrupy amide yielded 0·0021 g. of hydrazodicarbonamide. Assuming a yield of 40% of theoretical and that the mixture is composed of dimethyl pentose amides only, this result indicates the presence of some 14% of sugar not substituted on C_2: that is, 14% of a 3:4-dimethyl xylose present in the tiled syrup.

Attempted formation of Acetone Compounds.[11]

To a portion of the syrup (0·762 g.) in dry acetone (40 mls.) anhydrous cupric sulphate (4·0 g.) was added, followed by concentrated sulphuric acid (1 drop) and acetaldehyde (2 drops). The reactants were shaken at room temperature for 96 hours. The cupric sulphate was filtered off, the acid neutralised by shaking with moist calcium hydroxide, filtered and the acetone removed at 30°/15 mm. The residue was washed with water (2 × 25 mls.), filtered, and the

water removed under reduced pressure. The resulting syrup was tested for the presence of any acetone compounds on a micro-acetone apparatus. The percentage acetone found present was 1·56, which would correspond to the presence of 5% 1:2-isopropylidene-3:4-dimethyl xylose in the above product. The same result would have been given by 3 or 4-methyl xylose and all that may be said is that if 3:4-dimethyl xylose were present the amount could not have been significant.

Attempted formation of the Phenylhydrazide.

The syrupy lactone (0·061 g.) was dissolved in ether (1 ml.), and phenylhydrazine (0·060 g.) added. The mixture was refluxed for 30 minutes, the solvent removed in a vacuum desiccator, but there was no sign of crystallisation occurring.

Preparation of the Anilide.

The anilide was prepared from this syrupy fraction (0·229 g.) in the usual way. The crystalline material obtained on removal of the solvent in a vacuum desiccator was twice recrystallised from ethyl acetate, 0·090 g., m.p. 149°. This anilide was decomposed by heating with sulphuric acid (50 mls. of 0·1 N) for 1 hour at 70°. The solution was neutralised with barium carbonate, filtered, and the aqueous solution extracted with chloroform (to remove aniline), the aqueous layer being retained. The volume of the

solution was reduced at 40°/15 mm. and the resulting syrup dried with alcohol and benzene, giving 0·040 g. $\eta_D^{18°}$ 1·4740. On running a drop of a 5% aqueous solution of this syrup on a strip chromatogram[4] against standards, development showed the presence of 2:3 and 2:4-dimethyl xyloses as before, but the relative intensity of the spot due to the 2:3 derivative was greater than previously observed.

Investigation of the Combined Fractions C I and C II.

These combined fractions (2·475 g.) were distilled giving the distillate, 1·452 g., 110°-120°/0·01 mm. (bath temp.), $\eta_D^{22°}$ 1·4650, OMe, 41·6%, and a solid brown waxy residue remaining in the flask (0·9 g.).

Since this fraction appeared to be a mixture, an attempt at further separation was made by chromatographic adsorption on an alumina column.

[10] Column Dimensions. 28·5 cm. by 1·8 cm.

The combined fractions (1·452 g.) were dissolved in a 2:1 light petroleum (40°-60°) chloroform mixture (25 mls.), added to the column, and development commenced using this solvent.

No. of fraction.	Volume/ flask.	Wt. of syrup.	Refractice Index.	
I	200 mls.	0·064 g.	$n_D^{17°}$ 1·4602	
II	30 mls.	0·042 g.	$n_D^{18°}$ 1·4570	
III	60 mls.	0·049 g.	" 1·4576	Partly crystallised.
IV	80 mls.	0·109 g.	" 1·4580	
V	"	0·118 g.	" 1·4571	
VI	"	0·134 g.	" 1·4576	Combined
Total volume of 2:1 light petroleum chloroform mixture used was approx. 550 mls.				and
Weight of syrup recovered using this solvent was 0·516 g.				Hydrolysed.
Development was continued using chloroform.				
VII	80 mls.	0·196 g.	$n_D^{18°}$ 1·4570	
VIII	"	0·045 g.	$n_D^{17°}$ 1·4600	
IX	120 mls.	0·003 g.	-	
X	"	-	-	
Total volume of chloroform used was approx. 400 mls.				
Weight of syrup recovered using this solvent was 0·244 g.				
Development was continued using 4:1 chloroform methanol mixture.				
XI	80 mls.	0·170 g.	$n_D^{17°}$ 1·4678	
XII	"	0·362 g.	$n_D^{18°}$ 1·4700	Partly crystallised.
XIII	"	0·019 g.	" 1·4690	
Total volume of 4:1 chloroform methanol mixture used was approx. 250 mls.				
Weight of syrup recovered using this solvent was 0·551 g.				

Development was continued using methanol.

No. of fraction.	Volume/ flask	Wt. of syrup.	Refractive Index.
XIV	120 mls.	0·008 g.	$n_D^{18°}$ 1·4690
XV	"	0·026 g.	" 1·4678
XVI	"	0·011 g.	-

Total volume of methanol used was approx. 350 mls. Weight of syrup recovered using this solvent was 0·045 g. Recovery: 94%. All fractions were dried at 30°/0·5 mm. for 15 minutes.

Fractions II to VII were Combined and Hydrolysed.

These combined fractions (0·528 g.) were hydrolysed with nitric acid (20 mls. of 2%) for 5 hours at 100°. Found: $[\alpha]_D^{17°}$ +44·4°. A reducing syrup was recovered (0·483 g.) $n_D^{16°}$ 1·4688, OMe, 34·8%.

Anilide Formation.

This fraction (0·483 g.) was converted to the anilide in the usual way. On removal of the solvent a crystalline mass (0·410 g.) was obtained, m.p. 123°-136°.

On recrystallisation from alcohol:

Crop 1, 0·068 g., m.p. 163°, on subsequent recrystallisation had m.p. 172° unchanged on admixture with an authentic sample of 2:4:6-trimethyl galactose anilide.

$[\alpha]_D^{15°}$ (c, 0·3 in acetone). cf. (12).

Initial -96·9°, ½ hour -87·5°, 1½ hours -81·3°,

3 hours -53·1°, 5½ hours -26·3°, 8½ hours -6·3°, 11 hours +3·1°, 23 hours +31·3°, constant value.

2nd Crop, 0·098 g., m.p. 129°-32°. On subsequent recrystallisation had m.p. 143°, OMe, 24·4% ($C_{13}H_{19}O_4N$ (a dimethyl pentose anilide) requires OMe, 24·5%). Further recrystallisation caused the m.p. to drop.

3rd Crop, 0·103 g., m.p. 109°-118°, OMe, 26·3%. This fraction was decomposed by warming with sulphuric acid (5 mls. of 1%) and the free sugar recovered as described previously. This syrup was converted to the lactone and hence to the amide which, on removal of the solvent in a vacuum desiccator, crystallised. Recrystallisation from ethyl acetate gave a white crystalline solid, m.p. 128°-31°; mixed m.p. with an authentic sample of 2:3-dimethyl xylonamide was 127°-31°.

The partly crystalline fraction XII was tiled, the crystals recovered were recrystallised from light petroleum (60°-80°), and had m.p. 104°. $[\alpha]_D^{16°}$ (c, 2·1 in water) -33·6°, OMe, 49·7%. cf. (13). The syrupy portion was extracted from the tile with acetone (recovery 0·309 g.), and hydrolysed with nitric acid (10 mls. of 2%) for 5 hours at 100°. A syrup was obtained $[\alpha]_D^{15°}$ (c, 1·5 in water) +28·5°, $n_D^{14°}$ 1·4795. Part crystallisation occurred on standing and on recrystallisation from acetone the crystals had m.p. 126-7°.

Investigation of Fraction C III. 4·670 g., $n_D^{19°}$ 1·4746, OMe, 29·0%.

This fraction was purified by chromatographic adsorption on an alumina column.

[11] Column Dimensions. 17 cm. by 3·5 cm.

The syrup (4·670 g.) was dissolved in a mixture of 1:2 light petroleum chloroform (30 mls.), and added to the column which was developed using this solvent.

No. of fraction.	Volume/flask	Wt. of syrup	Refractive Index.
I	200 mls.	0·010 g.	-
II	100 mls.	0·077 g.	$n_D^{16°}$ 1·4617
III	125 mls.	0·030 g.	" 1·4577
IV	"	0·092 g.	" 1·4690

Total volume of 1:2 light petroleum chloroform mixture used was approx. 550 mls.

Weight of syrup recovered using this solvent was 0·209 g.

Development was continued using chloroform.

V	150 mls.	0·402 g.	$n_D^{15°}$ 1·4728	V to XIII Combined and Hydrolysed
VI	75 mls.	0·189 g.	" 1·4738	
VII	"	0·192 g.	" 1·4700	
VIII	"	0·122 g.	" 1·4720	

Total volume of chloroform used was approx. 375 mls.

Weight of syrup recovered using this solvent was 0·905 g.

Development was continued using 2:1 chloroform methanol mixture.

No. of fraction	Volume/flask	Wt. of syrup	Refractive Index.	
IX	75 mls.	0·115 g.	$n_D^{16°}$ 1·4720	V to XIII Combined and Hydrolysed.
X	40 mls.	1·462 g.	" 1·4735	
XI	"	0·934 g.	" 1·4686	
XII	"	0·437 g.	" 1·4700	
XIII	"	0·042 g.	$n_D^{17°}$ 1·4700	
XIV	75 mls.	0·040 g.	-	

Total volume of 2:1 chloroform methanol mixture used was approx. 300 mls.

Weight of syrup recovered using this solvent was 3·030 g.

Development was continued using methanol.

XV	70 mls.	0·022 g.	-	
XVI	"	0·021 g.	-	
XVII	"	0·037 g.	$n_D^{17°}$ 1·4743	
XVIII	150 mls.	-	-	

Total volume of methanol used was approx. 350 mls.

Weight of syrup recovered using this solvent was 0·080 g.

The alumina from the column was extracted on a Soxhlet extractor with methanol (200 mls.) for 4 hours. On removal of the solvent under reduced pressure a brittle yellow glass (0·369 g.) was recovered.

All fractions were dried at 30°/0·5 mm. for 15 minutes.

Recovery: 98%.

Fractions V to XIII were Combined and Hydrolysed.

These combined fractions (3·434 g.) were hydrolysed with nitric acid (75 mls. of 2%) for 5 hours

at 100°. Found: $[\alpha]_D^{15°}$ +29·1°. A reducing syrup was obtained (3·064 g.) having $n_D^{15°}$ 1·4840, OMe, 17·1%.

Periodic Acid Oxidation.

A portion of this syrup (0·0241 g.) was oxidised with periodic acid, as previously described (p.109), and the weight of dimedon complex obtained 0·0295 g. Assuming there are only monomethyl pentoses present, then the percentage not substituted on positions C_4 or C_5 is 69.

The remainder of this fraction was dissolved in the minimum quantity of alcohol, and the solvent slowly removed in a vacuum desiccator. In all, 6 crops of crystals were obtained, totalling 1·110 g. On a second recrystallisation from alcohol, the m.p./ was 135°-7°. Mixed m.p. with an authentic sample of 2-methyl xylose was unchanged. (Found: C, 44·3; H, 7·7; OMe, 19·9. $C_6H_{12}O_5$ requires C, 43·9; H, 7·4; OMe, 18·9%).

$[\alpha]_D^{16°}$ (c, 3·534 in water). cf. (14)

Initial -22·9°, 15 minutes -0·57°, 35 minutes +19·8°, 65 minutes +31·1°, 2 hours +34·2°, 4 hours +34·2°, 7 hours +34·5°, 24 hours +34·8°, constant value. From the rotational change this appeared to be the β form.

Periodic Acid Oxidation.

The crystalline sugar (0·0181 g.) was subjected to oxidation with periodic acid and the weight of

dimedon complex obtained 0·0278 g., corresponding to 86% of the sugar not substituted on positions C_4 or C_6.

Osazone Formation.

The crystalline sugar (0·101 g.) was used in the preparation of the phenylosazone. Phenylhydrazine hydrochloride (0·3 g.) was dissolved in water (5 mls.), filtered, and to the filtrate the crystals (0.101 g.) were added along with hydrated sodium acetate (0·5 g.) and a little sodium bisulphite. The reaction was kept at 100° for 1 hour. After filtering, a second crop was obtained by heating the filtrate for a further 20 minutes at 100° (Total 0·044 g.). On recrystallisation from aqueous alcohol had m.p. 144° (with decomp.). Mixed m.p. with an authentic sample of xylose phenylosazone (m.p. 156°-8°) was 154°-5°, OMe, 1·0%. It appeared that osazone formation was causing the removal of the methoxyl, presumably from C_2.

Anilide Formation.

The anilide was prepared from the crystalline sugar (0·088 g.) as described previously (p. 66). On removal of most of the alcohol in a vacuum desiccator, a crystalline product (0·073 g.) was obtained, having on recrystallisation from ethyl acetate and light petroleum (80°-100°), m.p. 125-6°. $[\alpha]_D^{18°}$ (c, 2·20 in ethyl acetate) +214°. (Found: C, 60·0; H, 7·1; N, 6·1 ; OMe, 12·8. $C_{12}H_{17}O_4N$ requires C, 60·3; H, 7·1 ; N, 5·9; OMe, 12·9%).

Lactone Formation.

The crystalline sugar (0·353 g.) was dissolved in water (10 mls.), and liquid bromine (1 ml.) added, the oxidation being kept at room temperature for 6 days. On freeing the solution from bromine by aeration it was found to be non-reducing to Fehling's solution. Lactonisation was carried out as previously described. Distillation gave a yellow syrup:

0·233 g., 155°-60°/0·01 mm. (bath temp.), $n_D^{14°}$ 1·4832.

The lactone crystallised completely in 7 days, the resulting product was tiled giving a white crystalline solid (0·155 g.), m.p. 66°-68°, OMe, 17·7%. Mixed m.p. with a sample of Mullan's lactone (m.p. 67°) was unchanged. (That is, Mullan's supposed 3:4-dimethyl xylonolactone was, in fact, impure 2-methyl xylonolactone).

$[\alpha]_D^{17°}$ (c, 1·134 in water):

Initial +100·6°, 1 hour +100·6°, 5 hours +97·9°, 23 hours +97·0°, 71 hours +92·6°, 167 hours +84·7°, 288 hours +81·1°, 383 hours +76·7°, 504 hours +74·1°.

From the rotational change this appeared to be a γ-lactone.

Titration of the Lactone with Sodium Hydroxide.

2 mls. of the above lactone solution (0·0227 g.) were titrated with N/40 sodium hydroxide. Titre of

0·02505 N sodium hydroxide required was 5·81 mls. (Calculated titre for $C_6H_{10}O_5$ was 5·60 mls.).

Amide Formation.

The amide was prepared from the lactone (0·060 g.) by addition of dry methyl-alcoholic ammonia (2 mls.). On removal of the solvent in a vacuum desiccator a crystalline product was obtained (0·040 g.) which was recrystallised from ethyl acetate, needles m.p. 96°-98°, $[\alpha]_D^{18°}$ (c, 1·95 in water) +52·4°. (Found: C, 40·0 ; H, 6·9 ; N, 8·3 ; OMe, 16·7 ; $C_6H_{13}O_5N$ requires C, 40·2; H, 7·3 ; N, 7·8 ; OMe, 17·3%).

Weerman Test.

A Weerman test was carried out on the crystalline amide (0·0331 g.) and simultaneously on a sample of gluconamide (0·0334 g.). The yield of hydrazodicarbonamide from gluconamide was 55% theoretical, while the crystalline amide gave no precipitate at all: that is, a negative Weerman indicating that C_2 was substituted with a methoxyl residue.

On removal of all the alcohol from C III a syrup (1·90 g.) was obtained which could not be induced to crystallise further. OMe, 18·9%.

Strip Chromatography.[4]

A spot of a 5% aqueous solution of this syrup was run on a strip chromatogram against standards. On development, one slightly elongated spot was obtained corresponding to the standard 2-methyl xylose. It was

found that an authentic mixture of 2 and 3-methyl xyloses could not be separated by strip chromatography, an elongated spot resulting.

Periodic Acid Oxidation.

A portion of this syrup (0·0227 g.) was oxidised with periodic acid and from the yield of dimedon complex obtained (0·0262 g.) the percentage monomethyl sugar not substituted on C_4 and C_5 was calculated to be 65.

Osazone Formation.

The phenyl osazone was prepared from the syrup (0·180 g.) as previously described, yielding a dark yellow solid (0·102 g.). This was dissolved in chloroform (10 mls.), and added to an alumina column (20 cm. by 1·8 cm.) and development continued using chloroform. The solvent was subsequently changed to a 98% benzene 2% methanol mixture, when the strongly adsorbed osazone band subdivided into two bands, the bottom of which was washed out, and on removal of the solvent under reduced pressure and recrystallisation from aqueous alcohol, had m.p. 166°-8°, 0·015 g., OMe, 8·7%. (Calculated for $C_{18}H_{22}O_3N_4$: OMe 9·1%).
Mixed m.p. with an authentic sample of 3-methyl xylose phenylosazone (m.p. 172°) was 166°. Mixed m.p. with an authentic sample of xylose phenylosazone (m.p. 156°-8°) was 143° (with decomp.).

Lactone Formation.

A portion of the syrup (0·302 g.) was converted to the lactone as previously described. On distillation a yellow syrup was obtained:
0·111 g., 160°-70°/0·05 mm. (bath temp.), $\eta_D^{13°}$ 1·4854.
No crystallisation occurred.

$[\alpha]_D^{17°}$ (c, 1·128 in water):
Initial +67·4°, 1 hour +67·4°, 5 hours +67·4°, 23 hours +66·5°, 95 hours +63·0°, 192 hours +54·6°, 287 hours +50·2°, 360 hours +46·7°, 528 hours +42·3°, constant value.

Titration of the Lactone with Sodium Hydroxide.

2 mls. of the above lactone solution (0·0226 g.) were titrated with N/20 sodium hydroxide. Titre of 0·0517 N sodium hydroxide required was 2·66 mls. (Calculated titre for $C_6H_{10}O_5$ was 2·70 mls.).

Amide Formation.

The amide was prepared from the lactone (0·090 g.) by addition of dry concentrated methyl-alcoholic ammonia (3 mls.) and the reaction kept at 0° for 3 days. A syrupy amide was obtained on removal of the solvent in a vacuum desiccator, 0·070 g., $\eta_D^{17°}$ 1·4850.

Weerman Test.

A Weerman test was carried out on this syrupy amide (0·0693 g.), and a control run simultaneously on gluconamide (0·0946 g.). The yield of hydrazodicarbon-

amide from the control (0·0130 g.) corresponded to 30% of theoretical; the yield from the syrupy amide was 0·0091 g. Assuming a 30% yield, this indicates that 49% of this syrupy amide was not substituted on C_2.

Examination of the Residue. (2·699 g.)

The dark-coloured glass showed no sign of crystallising after 6 months. A portion (0·5 g.) was dissolved in the minimum quantity of alcohol and the solvent slowly removed in a vacuum desiccator. The crystalline product obtained 0·100 g.) was tiled and had m.p. 154° after recrystallisation from ethyl acetate by addition of light petroleum (40°-60°). $[\alpha]_D^{17°}$ (c, 0·80 in water) -31·7°. Hydrolysis was effected with nitric acid (3 mls. of 2%) for 5 hours at 100°. A drop of a 5% aqueous solution of the resulting syrup was run on a strip chromatogram against standards, when only one spot was obtained that corresponding to the standard D-xylose.

The remainder of this fraction was hydrolysed with nitric acid (50 mls. of 2%) for 5 hours at 100°. A hard brittle mass was obtained: OMe, 8·1%. This mixture of sugars was dissolved in alcohol (75 mls.) and the solvent slowly removed in a vacuum desiccator, 3 crops of crystals being obtained (1·241 g.), m.p.141°. Mixed m.p. with an authentic specimen of D-xylose was unchanged.

Osazone Formation.

The osazone was prepared from the crystalline sugar (0·173 g.) as previously described. The osazone so obtained (0·180 g.) was recrystallised from aqueous alcohol and had m.p. 161-2°, mixed m.p. with an authentic sample of xylose phenylosazone was unchanged.

Strip Chromatography.[4]

A drop of a 5% aqueous solution of the crystalline sugar was run on a strip chromatogram against standards. On development, one spot appeared, corresponding to the standard D-xylose.

On complete removal of the solvent a syrupy portion was left which could not be induced to crystallise, and this was examined by strip chromatography. On development of the paper chromatogram, considerable tailing was noticed, in addition to which there were a number of sugars present. The bulk corresponded to the standard D-xylose, while there were moderately strong spots corresponding to the standard 2-methyl xylose, and an unidentified sugar, R.G. value 0·247 (mean of 7 values). A trace of galactose was also present. The sugar of R.G. value 0·247 did not correspond to authentic specimens of L-rhamnose, L-fucose, D-ribose, 2 or 6-methyl galactoses.

DISCUSSION Of RESULTS.

The classical method of complete methylation, followed by methanolysis and separation of the constituent sugars, was used in an attempt to determine the structure of the mucilaginous polysaccharide Plantago lanceolata. The "free acid" polysaccharide was acetylated with pyridine and acetic anhydride, the acetate being obtained in good yield (78%). The acetate was methylated three times with 30% sodium hydroxide solution and dimethyl sulphate, giving a chloroform soluble product (30% yield) which was fractionally precipitated from chloroform with light petroleum.

Part of the unfractionated methylated polysaccharide was subjected to a further methylation using thallium ethoxide, a reagent which had proved particularly effective in the methylation of complex polysaccharides;[15] however, in this case the latter method of methylation did not increase the methoxyl content of the polysaccharide already methylated three times by Haworth's method.

A number of viscosity determinations were carried out on the fractionated methylated polysaccharide dissolved in m-cresol. Whereas a branched chain structure was to be expected,[7] the viscosity

determinations seemed to indicate a chain of considerable length (η_{sp}/c 6·489). The specific viscosity of the fraction initially precipitated was found to be almost double that of the final fraction, and it was this first fraction which was examined in the third and most careful analysis (c).

The methylated polysaccharide was hydrolysed with 3% methyl-alcoholic hydrogen chloride, and the mixture so obtained was separated by (a) fractional distillation, (b) solvent extraction and (c) the non-reducing syrup obtained by the hydrolysis of the fractionated methylated polysaccharide separated by using a combination of fractional distillation and solvent extraction.

Separation by fractional distillation resulted in the products being collected in four main fractions. Fraction I: 44·5%; $n_D^{10°}$ 1·4480 ; OMe, 54·9%. Fraction II: 11·0%; $n_D^{10°}$ 1·4622 ; OMe, 47·1%. Fraction III: 9·7%; $n_D^{10°}$ 1·4762 ; OMe, 46·9%. Fraction IV: 11·8%; $n_D^{10°}$ 1·4762 ; OMe, 39·7%.

Fraction I was refractionated into three fractions, Ia. 17%, $n_D^{8°}$ 1·4447; Ib. 14%, $n_D^{8°}$ 1·4487, and Ic. 10%, $n_D^{8°}$ 1·4620, OMe 51·6%. Fraction Ia was shown to consist entirely of trimethyl methyl xylo-pyranosides, as it gave only 2:3:4-trimethyl xylose on hydrolysis. Fraction Ib consisted mainly of trimethyl

methyl xylosides, but also contained a small quantity of fully methylated methyl galactopyranosides, as on hydrolysis and anilide formation a small quantity of 2:3:4:6-tetramethyl galactose anilide was obtained.

From a consideration of the refractive index and methoxyl content Fraction Ic appeared to be a mixture, and an attempt was made to separate the glycosides present by chromatographic adsorption on an alumina column.(1) In the absence of any visible bands on the alumina the passage of the methylated glycosides was followed by the collection of a number of similar volumes of the developing solvent, and after removal of the solvent under reduced pressure, the determination of the refractive indexes of these fractions. The chromatographic fractions which showed little variation in refractive indexes were combined. Generally it was found that the more fully methylated the glycoside the less strongly it was adsorbed. In subsequent work (p.97), when separating a mixture of trimethyl methyl xylosides, tetramethyl methyl galactosides and dimethyl methyl xylosides, development using a solvent mixture of 4:1 light petroleum chloroform was found to be successful, although separation of these fully methylated glycosides was more satisfactory by fractional distillation (p.90). As a method of obtaining pure dimethyl and monomethyl xylosides, chromatographic adsorption is much superior

to fractional distillation.

Fractions II and III were also purified by chromatographic adsorption. Partial crystallisation occurred in the hydrolysed Fractions Ic_2 and IIa after several weeks, and the crystals recovered by tiling, then washed with light petroleum and chloroform. Ic_2 (0·010 g.) m.p. 103°-6°, IIa (0·015 g.) m.p. 103°-5°. Mixed m.p. with an authentic sample of 2:4-dimethyl xylose was 107°-8°. (In the chromatographic adsorption of Fraction Ic when development with chloroform was commenced, a small quantity of crystalline material was obtained, m.p. 75°; OMe, 43·5%, but owing to the lack of material, further investigation of this glycoside was prevented). When development with chloroform was commenced in Fraction II the first three fractions collected had refractive indexes ranging from 1·4500 to 1·4520. After hydrolysis, the free sugar had $[\alpha]_D^{15°}$ +97·5° and yielded a crystalline anilide, m.p. 193°-4°; mixed m.p. with an authentic sample of 2:3:4:6-tetramethyl galactose anilide was 190°-2°.

In Fraction III the first four fractions collected, after development with chloroform was commenced, were combined and hydrolysed. On anilide formation a small quantity of 2:4:6-trimethyl galactose anilide was isolated, m.p. 171°-2°; OMe, 32·0%, mixed m.p. with an authentic sample being unchanged. The

above separation by fractional distillation had shown the mixture of glycosides obtained by methanolysis of the fully methylated mucilage to contain 2:3:4-trimethyl methyl xylosides (ca. 31%), 2:3:4:6-tetramethyl methyl galactosides, 2:4:6-trimethyl methyl galactosides and 2:4-dimethyl methyl xylosides, in addition to less fully methylated glycosides (ca. 23%). The dimethyl methyl pentose fraction amounted to some 30% of the whole.

Separation by solvent extraction depends on the fact that fully methylated sugars are much more readily extracted from water with light petroleum (38°-40°) than are partially methylated sugars. The partition coefficient of fully methylated glucose between light petroleum and water is about 0·1, while the corresponding figure for trimethyl methyl glucoside is less than 0·01, the ratio between these figures being such that it is possible to develop a method for the separation of the sugar derivatives by a continuous extraction process.(2) An aqueous solution of the non-reducing mixture of sugars obtained on methanolysis of the unfractionated methylated mucilage was extracted for varying lengths of time with the solvents, light petroleum (38°-40°) and chloroform. The more fully methylated glycosides were removed with light petroleum, while removal of the less fully methylated fractions was accomplished with chloroform.

Three fractions were obtained on extraction with

light petroleum (38°-40°). Fraction P I; 37·4%, $n_D^{15°}$ 1·4450, 3 hours. Fraction P II; 5·7%, $n_D^{15°}$ 1·4497, 7 hours. Fraction P III: 2·9%, $n_D^{15°}$ 1·4555, 10 hours. A further three fractions were obtained on extraction with chloroform. Fraction C I: 12·6%, $n_D^{15°}$ 1·4633, ½ hour. Fraction C II: 26·6%, $n_D^{15°}$ 1·4654, 3½ hours. Fraction C III: 8·7%, $n_D^{15°}$ 1·4738, 23½ hours. Residue: 4·5%, $n_D^{11°}$ 1·4800. Recovery: 98%. The most notable advantage which this method has over fractional distillation is the high percentage recovery. Fraction P I was refractionated with light petroleum giving two fractions, and on further extraction with chloroform, a third: P Ia. 18·7%, $n_D^{16°}$ 1·4456, P Ib. 5·4%, $n_D^{15°}$ 1·4431. P Ic. 5·4%, $n_D^{11°}$ 1·4448.

On removal of the solvent from fraction P Ia, a small quantity of crystalline material, m.p. 45°-8°, was obtained which proved to be insoluble in water and therefore did not appear to be glycosidic in character. Indeed, it may not have been unrelated to the waxy product obtained on hydrolysis of all the light petroleum fractions. There is a possibility that this wax may have been associated with the polysaccharide in its natural state, being split off during methanolysis, while in previous separations by fractional distillation it had remained in the flask, charring at the higher temperatures. On removal of the waxy material after hydrolysis of Fraction P Ia, the remaining syrup

crystallised completely, having m.p. 87°-90° on recrystallisation from ether and it appeared that this fraction, as did Fraction P Ib, consisted entirely of trimethyl methyl xylopyranosides. The syrup obtained on hydrolysis of Fraction P Ic crystallised only partially, and the mother liquor on anilide formation gave a good yield of 2:3:4:6-tetramethyl galactose anilide, m.p. 195°. In the above separation it appeared that the fully methylated xyloside (OMe, 60·1%) was extracted in preference to the fully methylated galactoside (OMe, 62%).

Fraction P II, on hydrolysis, gave a syrup which did not crystallise, and on conversion to the anilide yielded 2:3:4:6-tetramethyl galactose anilide, m.p. 195°.

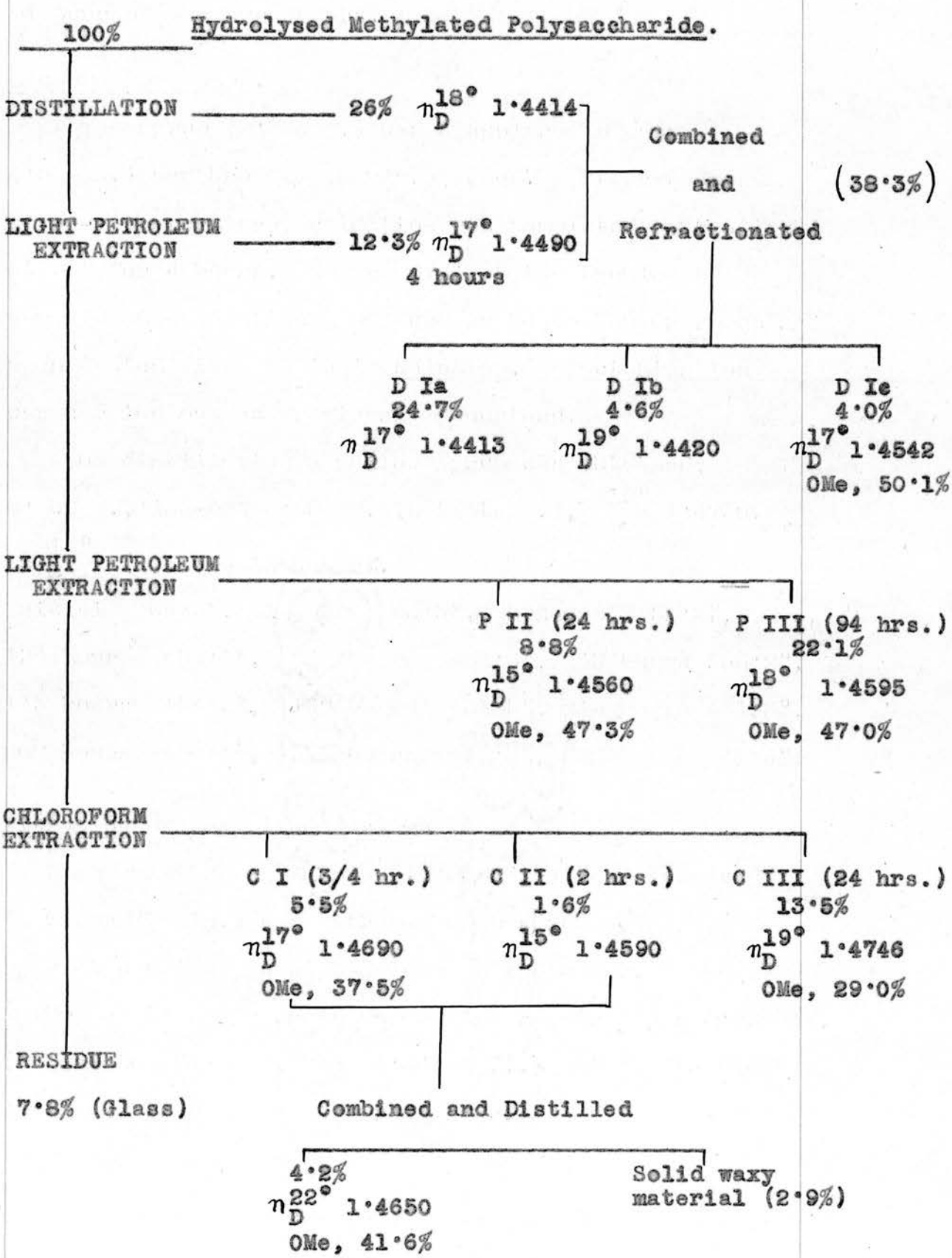
Fraction P III was examined in conjunction with Fraction C Ia, obtained from C I by distillation from a waxy material (2·7%). These combined fractions were purified by chromatographic adsorption, and when development was commenced with chloroform, seven fractions were collected having refractive indexes ranging from 1·4570 to 1·4581 (9·8%), which were combined and hydrolysed. A syrup was obtained (OMe, 34·6%) which crystallised partly on standing, and after tiling and washing with light petroleum followed by chloroform, had m.p. 107°-9°, unchanged on admixture with an authentic sample of 2:4-dimethyl xylose.

Fraction C II was also separated into two portions by fractional distillation, C IIa: 16·1%, $n_D^{16°}$ 1·4626, OMe, 42·1; C IIb: 7·8%, $n_D^{16°}$ 1·4661, OMe, 40·7%. Fraction C IIa was further purified by chromatographic adsorption, and the first seven fractions (refractive indexes from 1·4580 to 1·4593) collected after commencing development with chloroform. These combined fractions (9·6%) were hydrolysed to give a syrup, OMe 35·6%, which crystallised partly, and after tiling and washing as before had mp. 109°, unchanged on admixture with 2:4-dimethyl xylose. Fractions XVII and XVIII obtained on development with methanol (refractive index 1·4722) crystallised on standing and had m.p. 101°-3°, $[\alpha]_D$ -56·4°. Hydrolysis, followed by examination by paper chromatography, showed the presence of 2-methyl xylose, and a trace of glucose, and so it appeared that the crystalline material was in fact 2-methyl-β-methylpyranoside.(14) Fraction C IIb was, in turn, passed through an alumina column, and the six fractions (refractive indexes ranging from 1·4572 to 1·4608) obtained on development with chloroform were collected (4·6%) and hydrolysed, giving a syrup which did not crystallise. Although oxidation to the lactone and subsequent amide formation were not carried out on this sample, a similar non-crystalline fraction (P IIIb p. 107) was shown to consist mainly of 2:3-dimethyl xylose.

A portion of C III was methylated with silver oxide and methyl iodide, hydrolysis of the fully

methylated product giving only 2:3:4-trimethyl xylose, OMe, 47·2%; m.p 87°-90°, indicating that C III contained only xylose derivatives. An attempt was made to detect the presence of ester groupings in this fraction by heating with N/20 sodium hydroxide for one hour at 100° and estimating the alkali used by back-titration with hydrochloric acid. The value obtained (2·6%) indicated that only a very small proportion of such linkages existed. Separation by solvent extraction had shown the mixture of glycosides, obtained by methanolysis of the fully methylated polysaccharide, to contain 2:3:4-trimethyl methyl xylosides (ca. 35%), 2:3:4:6-tetramethyl methyl galactosides, 2:4-dimethyl methyl xylosides and 2-methyl-β-methyl xyloside. The dimethyl methyl pentose fractions amounted to some 32% and the less fully methylated glycosides ca. 17%.

A third and final fractionation of the products of methanolysis of the fractionated methylated mucilage (η_{sp}/c' = 125) was carried out, in which a combination of fractional distillation and solvent extraction was embodied.


100% Hydrolysed Methylated Polysaccharide.
DISTILLATION
26% $n_D^{18°}$ 1·4414
Combined and Refractionated (38·3%)
LIGHT PETROLEUM EXTRACTION
12·3% $n_D^{17°}$ 1·4490
4 hours
D Ia
24·7%
$n_D^{17°}$ 1·4413
D Ib
4·6%
$n_D^{19°}$ 1·4420
D Ie
4·0%
$n_D^{17°}$ 1·4542
OMe, 50·1%
LIGHT PETROLEUM EXTRACTION
P II (24 hrs.)
8·8%
$n_D^{15°}$ 1·4560
OMe, 47·3%
P III (94 hrs.)
22·1%
$n_D^{18°}$ 1·4595
OMe, 47·0%
CHLOROFORM EXTRACTION
C I (3/4 hr.)
5·5%
$n_D^{17°}$ 1·4690
OMe, 37·5%
C II (2 hrs.)
1·6%
$n_D^{15°}$ 1·4590
C III (24 hrs.)
13·5%
$n_D^{19°}$ 1·4746
OMe, 29·0%
RESIDUE
7·8% (Glass)
Combined and Distilled
4·2%
$n_D^{22°}$ 1·4650
OMe, 41·6%
Solid waxy material (2·9%)

Fractions IA and IB of the fractionated methylated mucilage were combined and hydrolysed with 3% methyl-alcoholic hydrogen chloride for 22 hours and the non-reducing mixture of glycosides obtained transferred to a vacuum-jacketed Claisen flask, and the fully methylated fractions distilled off. Three fractions were thus obtained: (I) 15·0%, $n_D^{18°}$ 1·4410; (II) 9·4%, $n_D^{18°}$ 1·4414; (III) 1·8%, $n_D^{18°}$ 1·4418. The undistilled glycosides were then extracted with light petroleum, giving Fraction P I , 12·3%, $n_D^{17°}$ 1·4490, 4 hours, which was combined with the above three fractions, and the whole redistilled to give Fractions D Ia, 24·7%, $n_D^{17°}$ 1·4413 ; D Ib, 4·6%, $n_D^{19°}$ 1·4420 ; D Ic, 4·0%, $n_D^{17°}$ 1·4542, OMe, 50·1%. The extraction with light petroleum was continued to give a further two fractions: Fraction P II. 8·8%, $n_D^{15°}$ 1·4560, OMe, 47·3%, 24 hours, and Fraction P III. 22·1%, $n_D^{18°}$ 1·4595, OMe, 47·0%, 94 hours. A further three fractions were obtained with chloroform: Fraction C I. 5·5%, $n_D^{17°}$ 1·4690, OMe, 37·5%, 3/4 hour. Fraction C II. 1·6%, $n_D^{15°}$ 1·4590, 2 hours. Fraction C III. 13·5%, $n_D^{19°}$ 1·4746, OMe, 29·0%, 24 hours. The Residue was a brown glassy solid (7·8%). Fractions C I and C II were combined and distilled to give a syrupy fraction, 4·2%, $n_D^{22°}$ 1·4650, OMe, 41·6%, and a solid waxy residue (2·9%).

Since a trace of L-arabinose had been

detected in the autohydrolysis of the "free acid" mucilage (Part I, p.46), it may have appeared in the products of methanolysis, possibly as the 2:3:5-trimethyl methyl arabofuranoside in conjunction with the fully methylated xylosides. Fractions D Ia and D Ib were combined and hydrolysed. The resulting crystalline 2:3:4-trimethyl xylose was washed with ether, the solvent removed and the syrup obtained allowed to crystallise. This process was continued until a syrup was obtained which could not be induced to crystallise, the fully methylated derivatives separated by chromatographic adsorption and converted to the lactone, when partial crystallisation as the 2:3:4-trimethyl xylonolactone (m.p. 47°-8°) occurred. The remaining syrupy lactone showed $[\alpha]_D$ + 6·4° to +12·7° (64 hours). Since 2:3:5-trimethyl arabonolactone has $[\alpha]_D$ -42° to -37° (88 hours)[5] it did not appear that this arabinose derivative was present in the fully methylated fractions. The crystalline 2:3:4-trimethyl xylose which had been obtained from D Ia and D Ib had m.p. 88°-90°, $[\alpha]_D$ + 53·8° to +20·2° (1 hour), and therefore appeared to be present in the α form. The crystalline 2:3:4-trimethyl - δ - xylonolactone, m.p. 53°, prepared from the crystalline sugar had $[\alpha]_D$ +1·3° to +19·1° (120 hours). The 2:3:4-trimethyl xylose anilide was also prepared from the crystalline sugar and had m.p. 100°-101°.

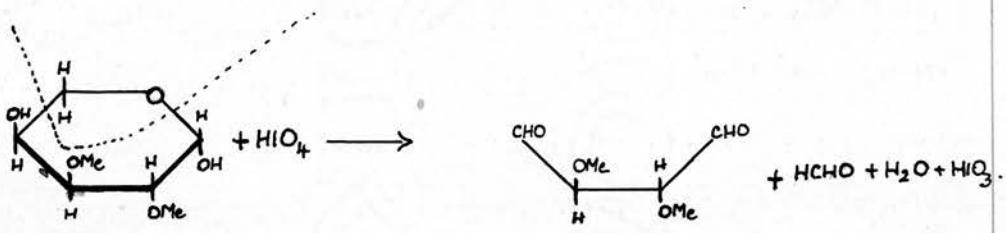
Fraction D Ic was purified by chromatographic adsorption, and Fractions IV to XI (refractive indexes from 1·4480 to 1·4489), obtained on development with 4:1 light petroleum chloroform, were collected. These combined fractions (3·3%) were hydrolysed and formed the 2:3:4:6-tetramethyl galactose anilide when standing at room temperature with an alcoholic solution of aniline; m.p. 192°-3°. On development with chloroform, Fractions XII to XIV (refractive indexes from 1·4520 to 1·4547) were combined and hydrolysed. Anilide formation gave a good yield of 2:3:4:6-tetramethyl galactose anilide, m.p. 192° $[\alpha]_D$ -74·9°, OMe, 37·1%. From the yield of tetramethyl galactose anilide obtained, and assuming a 90% recovery as the anilide, the percentage 2:3:4:6-tetramethyl methyl galactoside present was calculated to be approx. 3%. On continuing development with chloroform, Fractions XV to XVIII (refractive indexes from 1·4550 to 1·4560) were combined (4·0%) and hydrolysed. The resulting syrup crystallised partly, and on recrystallisation from acetone by addition of light petroleum (80°-100°), had m.p. 108°-10°. $[\alpha]_D$ +3·8° (17 minutes) to +22·7° (24 hours); that is, the crystalline sugar appeared to be the β form of 2:4-dimethyl xylose.

Fraction P II was passed through an alumina column, and Fractions III to IX (refractive indexes from 1·4550 to 1·4565) obtained on development with 2:1

light petroleum chloroform were combined (6·2%) and hydrolysed. The resulting syrup crystallised partly to give crystals of 2:4-dimethyl xylose, m.p. 108°-10°.

Fraction P III was also chromatographed, and on development with 1:1 light petroleum chloroform, Fractions III to X (refractive indexes 1·4569 to 1·4582) were combined (13·4%) and hydrolysed, the resulting syrup crystallising partly to give crystals of 2:4-dimethyl xylose, m.p. 108°-10°. When development was continued using chloroform, Fractions XI to XVI (refractive indexes 1·4552 to 1·4567) were combined (4·8%) and hydrolysed to give a syrup which would not crystallise. It was thought that since this syrup showed no sign of crystallising, whereas the other dimethyl fractions had, it might contain a high proportion of the 3:4-dimethyl xylose recorded as being present in this mucilage.(7) On lactone formation and distillation, two fractions were collected, one being set aside to see if crystallisation would occur, the other being used in following the rotational change and in amide formation. The lactone showed $[\alpha]_D$ +63·1° to 54·4 (384 hours) and appeared therefore to possess a 1:4 ring. The amide crystallised and had m.p. 132°-3° $[\alpha]_D$ +49·7°; mixed m.p. with an authentic sample of 2:3-dimethyl xylonamide was unchanged. That this was, in fact, the 2:3-dimethyl xylose derivative was further supported by periodic acid oxidation of the amide from

which a 97% yield of formaldehyde was obtained. Periodic acid oxidation and subsequent formaldehyde detection and estimation[10] offers a quick and reliable method for the detection of the 2:3-dimethyl xylose



2:3-dimethyl xylose

It has beenfound, however, that certain methylated sugars (e.g., 3-methyl glucose), when oxidised with periodic acid, do not give quantitative yields of formaldehyde, and that the rate of reaction is somewhat slower than usual.[16]

The crystalline 2:4-dimethyl xylose was oxidised with periodic acid, and substitution on C_4 confirmed by the absence of any formaldehyde-dimedon complex. The syrupy lactone was prepared from the crystalline sugar and had $[\alpha]_D$ -4·7° to +26·4° (48 hours), the rotational change indicating a 1:5, or δ , ring. The lactone yielded a syrupy amide which gave a negative Weerman test, thus confirming the presence of a methoxyl residue on C_2. Some difficulty was encountered in obtaining a sample of 2:4-dimethyl xylose

anilide which melted at the value quoted[3], it not being found possible to raise the m.p. above 161°, as decomposition of the anilide appeared to occur on recrystallisation

The syrupy dimethyl pentose fraction which was recovered from the tile with acetone, after the removal of the crystalline 2:4-dimethyl xylose, was then investigated. Osazone formation gave a product with OMe, 6·6%, indicating that C_2 which was involved in osazone formation was previously occupied by a methoxyl group which had been removed during compound formation. ($C_{18}H_{22}O_3N_4$ requires OMe, 9·3%). Periodic acid oxidation appeared to indicate that only 9·3% of the sugars present were not substituted on positions C_4 and C_5, although strip chromatography showed the presence of 2:4 and 2:3-dimethyl xyloses in approximately 40/60 proportion. Oxidation gave a syrupy lactone which had $[\alpha]_D$ +65·5°, +50·1° (17½ hours), +55·2° (43 hours) and 48·6° (600 hours). Calculated from the initial rotations of 2:3[8] and 2:4-dimethyl xylonolactones[3], the above mixture was calculated to contain 57% of 2:3, and 43% of 2:4-dimethyl xylonolactone: that is, assuming that only these two lactones were present. This assumption is not altogether valid, however, as a Weerman test showed that the amide contained 14% of a sugar not substituted on C_2. This result may be attributed to the presence of 3:4-dimethyl xylose, or to

3-methyl or 4-methyl xyloses in the mixture. Of these the presence of 3-methyl xylose was established in Fraction C III (p. 127). Titration of the mixed lactones with N/20 sodium hydroxide showed the presence of 10% free sugar and may have accounted for the sudden drop initially, +65·5° to 50·1° (17½ hours), and the sugar judged most likely to be present was 2:4-α-dimethyl xylose. It was thought that partially methylated xyloses not substituted on C_2, such as 3:4-dimethyl xylose, might be quantitatively detected as the acetone compound, as they would form the 1:2-isopropylidene derivative, whereas 2:3 and 2:4-dimethyl xyloses would not engage in compound formation with acetone. Micro-detection of acetone showed the presence of ca. 5% of a monacetone xylose in the product obtained, but it could not be decided whether it was a mono or a dimethyl derivative. The anilide formed from the syrupy fraction was twice recrystallised from ethyl acetate, m.p. 149°. Decomposition with sulphuric acid and examination by strip chromatography showed that the percentage of 2:3-dimethyl xylose had increased in the mixture over the 60/40 ratio previously recorded.

The combined fractions C I and C II were chromatographed, and after development with 2:1 light petroleum chloroform, Fractions II to VII (refractive indexes 1·4570 to 1·4580) were combined (2·1%) and hydrolysed. The resulting syrup was converted to the

anilide which on recrystallisation from alcohol gave three main crops of crystals. Crop I yielded 2:4:6-trimethyl galactose anilide, m.p. 172°, $[\alpha]_D$ -96·9° to +31·3° (23 hours). Crop III which had m.p. 109°-18°, OMe, 26·3%, was decomposed with sulphuric acid, and on conversion to the lactone and hence to the amide, gave a crystalline product, m.p. 128°-31°, unchanged on admixture with an authentic sample of 2:3-dimethyl xylonamide. Fraction XII (refractive index 1·4700) crystallised partly, and on recrystallisation from light petroleum had m.p. 104°, $[\alpha]_D$ -33·6°, OMe, 49·7%. This appeared to be the crystalline 2:4:6-trimethyl-β-methyl galactoside. The syrupy glycoside recovered from the tile was hydrolysed and the resulting syrup ($n_D^{14°}$ 1·4795) crystallised partly, m.p. 126°-7°, on recrystallisation from acetone, and was in all probability the crystalline 2-methyl xylose.

Fraction C III was chromatographed, and Fractions V to XIII (refractive indexes 1·4686 to 1·4738), obtained on development with chloroform, were combined (11·3%) and hydrolysed. A periodic acid oxidation on the resulting syrup showed that 69% of this fraction was not substituted on C_4 and C_5. Attention may be drawn to the fact that 3-methyl xylose (which was shown to be present in the fraction) does not give the theoretical yield of formaldehyde[16] on oxidation with periodic acid, and its presence would

account for the slightly low value obtained. On crystallisation from alcohol, a crystalline sugar was obtained (3·2%), m.p. 135°-7°, $[\alpha]_D$ -22·9° to +34·8° (24 hours). Mixed m.p. with an authentic sample of 2-methyl xylose was unchanged, while the rotational change indicated the sugar was present as the β form. Periodic acid oxidation gave 86% of the theoretical yield of formaldehyde. The presence of a methoxyl residue on C_2 was supported by osazone formation, when a product was obtained, having OMe, 1·0%. The anilide was prepared and had m.p. 125°-6°, $[\alpha]_D$ +214°. The crystalline sugar was oxidised to the lactone which crystallised and had m.p. 66°-8°. Mixed m.p. with a sample of Mullan's[7] supposed 3:4-dimethyl xylonolactone (m.p. 67°) was unchanged. (That is, Mullan's 3:4-dimethyl xylonolactone was, in fact, 2-methyl xylonolactone). The crystalline lactone had $[\alpha]_D$ +100·6° to +74·1 (504 hours), which indicated the presence of a 1:4 ring. The lactone, in turn, yielded a crystalline amide, m.p. 96°-8°, $[\alpha]_D$ +52·4°, which gave a negative Weerman test.

The syrupy fraction remaining after the removal of the crystalline 2-methyl xylose was examined using strip chromatography,[4] when one spot corresponding to 2-methyl xylose was obtained. However, it was found that an authentic mixture of 2 and 3-methyl xyloses could not be separated by this method, and

therefore the presence of one spot did not necessarily exclude the presence of 3-methylxylose in this syrupy fraction. Periodic acid oxidation indicated that 65% of the sugars present were not substituted on C_4 and C_5, and the presence of 3-methyl xylose was confirmed by osazone formation. The osazone prepared from the syrupy fraction was chromatographed and the lower yellow band proved to be 3-methyl xylose phenylosazone, m.p. 166°-8°, OMe, 8·7%. Mixed m.p. with an authentic sample of 3-methyl xylose phenylosazone (m.p. 172°) was unchanged, while admixture with xylose phenylosazone (m.p. 156°-8°) was 143°. The mixed lactones were prepared from the syrupy fraction and showed no sign of crystallising, having $[\alpha]_D$ +67·4° to +42·3° (528 hours), and gave a syrupy amide which, when subjected to a Weerman test, indicated that 49% of this mixture was not substituted on C_3.

The residual dark-coloured glass yielded a small amount of crystalline material, m.p. 154°, $[\alpha]_D^{17°}$ -31·7°, which on hydrolysis and examination by strip chromatography[4] showed the presence of D-xylose only, and it was concluded that the crystals were β-methylxyloside.[14]

The remaining syrup was hydrolysed, and on crystallisation from alcohol gave D-xylose (3·6%), m.p. 141°, unchanged on admixture with an authentic sample. The osazone prepared from the crystalline

sugar had m.p. 161°-2° unchanged on admixture with an authentic sample of xylose phenylosazone. Further confirmation as to the nature of this sugar was furnished by strip chromatography when the sample corresponded to the standard D-xylose.

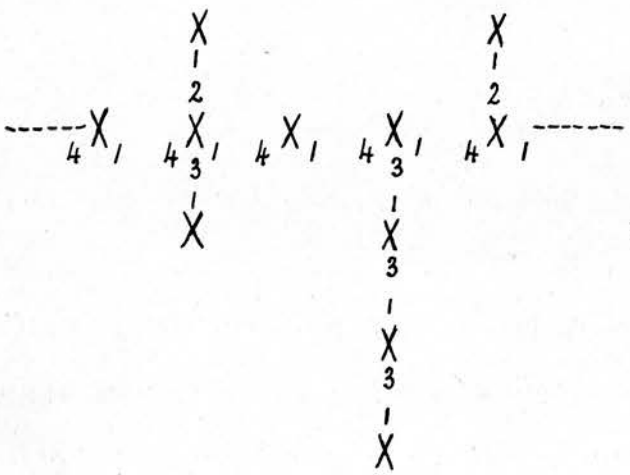
On complete removal of the crystalline xylose a syrup remained which was examined by strip chromatography. A strong spot appeared corresponding to D-xylose with spots of lesser intensity corresponding to 2-methyl xylose, and a sugar of RG value 0·247, thought to be a monomethyl hexose. A trace of galactose was also present.

A study of the results obtained shows the products of hydrolysis to contain the following glycosides in the percentages indicated:- 2:3:4-trimethyl methyl xylopyranosides (37%), 2:3:4:6-tetramethyl methyl galactopyranosides (2·4%), 2:4:6-trimethyl methyl galactopyranosides (1·2%), 2:4-dimethyl methyl xylopyranosides (16·1%), 2:3-dimethyl methyl xylopyranosides (15·5%), 2-methyl methyl xylopyranosides (8·4%), 3-methyl methylxylopyranosides (5·0%), methylxylopyranosides (6·8%), and a monomethyl hexose (1%) not identified. 2·5% of a waxy material was also obtained but no conclusion was reached as to whether this was an integral part of the molecule or not. A quantitative separation of this complicated mixture of glycosides was somewhat difficult, and any structural formula

submitted on the basis of the relative proportions of the sugars present must be regarded with some reserve. If the repeating unit of the polysaccharide is considered to have one D-galactose residue as an end-group and a second D-galactose residue linked 1:3 in its structure, then the constitution of the mixture obtained on methanolysis could be represented by trimethyl methyl xylopyranosides (15 mols.), tetramethyl methyl galactopyranosides (1 mol.), 2:4:6-trimethyl methyl galactosides (ca. 1 mol.), 2:4-dimethyl methyl xylosides (7 mols.), 2:3-dimethyl methyl xylosides (7 mols.), 2-methyl methyl xylosides (4 mols.), 3-methyl methyl xylosides (2 mols.) and methylxylosides (4 mols.). The various permutations and combinations of linkages in such a large repeating unit (41 mols.) are so great as to make a discussion of a possible constitution on that basis unprofitable, even if the accuracy of the molecular proportions could be relied upon. However, if we consider the galactose residues and the unidentified monomethyl hexose residues not to be fundamental to the repeating unit, and consider the structure to be composed entirely of D-xylose, the matter can be somewhat simplified. Converting the percentages to 100 for the xylose units we have: trimethyl methyl xylopyranosides (38·8%), 2:4-dimethyl methyl xylosides (16·9%), 2:3-dimethyl methyl xylosides (16·2%), 2-methyl methyl xylosides (8·8%), 3-methyl methyl xylosides (5·3%) and

methylxylosides (7·1%). It will be observed that the very high proportion of fully methylated xylose found can only be explained by taking into account the unsubstituted methylxylosides isolated on methanolysis, since the monomethyl xylosides, which can have only one fully methylated xylose addendum, are insufficient in quantity. There are, therefore, no grounds for the belief that the free xyloside is produced either as a result of incomplete methylation or of demethylation during methanolysis.

For such a repeating unit as the following



Where

X = D-xylopyranose.

we should obtain on methanolysis:

4 molecules of trimethyl methyl xylopyranosides (39·0%)

2 molecules of 2:4-dimethyl methyl xylosides (18·2%),

2 molecules of 2:3-dimethyl methyl xylosides (18·2%),

1 molecule of 2-methyl methyl xylosides (8·4%),

1 molecule of 3-methyl methyl xylosides (8·4%) and

1 molecule of methylxylosides (7·8%). Analysis of

such a mixture would provide methylxylosides in the respective percentages, indicated in parenthesis.

The agreement is not unreasonable and it may be suggested, therefore, that the fundamental repeating unit could be represented in some such way as indicated above, although the order in which the units are arranged is, of course, unknown.

Periodic acid oxidation of the "free acid" mucilage released 2 molecules of formic acid/7 sugar residues (p. 44). Such a unit as depicted above would release 2 molecules of formic acid/5·5 sugar residues. In this repeating unit no account is taken of the uronic acid residues which could not be detected among the products of methanolysis, and it was assumed that decomposition of this residue occurred either during acetylation[17] or methylation.

By joining 4 such repeating units together and adding 2 galactose residues, some approximation to the structure of this whole repeating unit, neglecting the uronic acid complex, might be reached.

SUMMARY.

1. Acetylation of the "free acid" polysaccharide, followed by methylation, gave a methylated derivative of OMe 35% which was not increased by subsequent methylation by thallium ethoxide. Fractionation gave two fractions I and II.

2. The products of methanolysis of the unfractionated methylated mucilage were separated (a) by fractional distillation, and (b) by solvent extraction. Both methods were combined in the separation of the fractionated mucilage from which the following sugars were isolated:

2:3:4-trimethyl methyl xyloside (37%), identified as the crystalline sugar m.p. 88°-90°, $[\alpha]_D$ +53·8° to 20·2° (1 hour), the crystalline δ-lactone m.p. 53°, $[\alpha]_D$ +1·3° to +19·1° (120 hours), and crystalline anilide m.p. 100°-101°.

2:3:4:6-tetramethyl methyl galactoside (2·4%), identified as the crystalline anilide m.p. 192°, $[\alpha]_D$ -74·9°.

2:4:6-trimethyl methyl galactoside (1·2%), identified as the 2:4:6-trimethyl β-methyl galactoside m.p. 104°, $[\alpha]_D$ -33·6°, and the crystalline anilide m.p. 172°, $[\alpha]_D$ -96·9° to +31·3° (23 hours).

2:3-dimethyl methyl xyloside (15·5%) identified as the crystalline amide m.p. 132°-3°, $[\alpha]_D$ +49·7°,

formaldehyde liberation on periodic acid oxidation, and negative Weerman. The syrupy γ-lactone had $[\alpha]_D$ +63·1° to 54·4° (384 hours).

2:4-dimethyl methyl xyloside (16·2%) was identified as the crystalline sugar m.p. 108°-10°, $[\alpha]_D$ +3·8° (17 minutes) to +22·7° (24 hours), negative periodic acid oxidation and negative Weerman.

The syrupy δ-lactone had $[\alpha]_D$ -4·7° to +26·4° (48 hours).

2-methyl methyl xyloside (8·2%) identified as the crystalline sugar m.p. 135°-7°, $[\alpha]_D$ -22·9° to +34·8° (24 hours), crystalline lactone m.p. 66°-68° $[\alpha]_D$ +100·6° to +74·1° (504 hours) and crystalline amide m.p. 96°-8° $[\alpha]_D$ +52·4°.

Periodic acid oxidation gave formaldehyde and the amide a negative Weerman. The anilide had m.p. 125°-6° $[\alpha]_D$ +214°.

3-methyl methyl xyloside (5·0%) identified as the osazone m.p. 166°-8°, positive Weerman and positive periodic acid oxidation.

Methylxyloside (6·8%) identified as the β-methyl-xyloside m.p. 154°, $[\alpha]_D$ -31·7°, crystalline sugar m.p. 141° and osazone m.p. 161°-2°.

A monomethyl hexose of R.G. value 0·247 was not characterised, and the presence of a 3:4-dimethyl xylose was not established. No arabinose derivative was detected.

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PART III.

The Study of the Aldobiuronic Acid.

Hydrolysis of the "free acid" Polysaccharide.

The polysaccharide (117 g.) was hydrolysed with oxalic acid (2 ℓ. of 3%) for 20 hours at 100°. The insoluble residue (11·5 g.) was filtered off and the filtrate neutralised with barium carbonate in the presence of charcoal. The mixture was kept at 100° for 30 minutes and the filtrate evaporated to 200 mls. at 40°/15 mm. This solution (200 mls.) was poured slowly with constant stirring into methanol (4 ℓ.), yielding a white precipitate of barium salt (10·3 g.), which was removed by centrifuging and washed with methanol and ether. This salt was hydrolysed with oxalic acid (200 mls. of 3%) for a further 6 hours at 100°, and the barium salt separated as before (6·9 g.). % CO_2 , 6·9 $(C_{12}H_{21}O_{11})_2Ba$ requires % CO_2 , 10·7 ; % Ba, 16·7. This barium salt (6·9 g.) was rehydrolysed with sulphuric acid (100 mls. of 4%) for 6 hours at 100°, and the barium salt separated (4·0 g.) had $[\alpha]_D^{17°}$ +46·9° ; % CO_2 , 7·3 ; % Ba, 10·1. The methyl alcoholic solution obtained after the removal of the barium salt was evaporated under reduced pressure to give a yellow syrup (0·788 g.). A spot of a 5% aqueous solution of this syrup was run on a strip chromatogram against standards, and on development D-xylose, L-arabinose and L-rhamnose were shown to be present with a lesser quantity of D-galactose. The presence of arabinose was confirmed

by the preparation of the arabinose diphenylhydrazone (m.p. 204°), and the weight of this derivative corresponded to the presence of 35·4% arabinose in the mixture. After standing for several weeks this syrup crystallised partly, the crystals recovered, m.p. 90°-94° (0·034 g.), when run on a strip chromatogram corresponded to the standard L-rhamnose. The syrup remaining still showed the presence of L-rhamnose when examined by strip chromatography.

The barium salt (4·0 g.) was again subjected to hydrolysis with sulphuric acid (50 mls. of 6%) for 6 hours at 100°. The barium salt (2·69 g.) was recovered as described above and had $[\alpha]_D^{17°}$ (c, 1·04 in water) +49·7° ; % CO_2, 9·4 ; % Ba, 15·4. On evaporation of the alcoholic solution under reduced pressure a syrup was obtained (0·07 g.) which on examination by strip chromatography showed the presence of L-arabinose with traces of D-xylose and D-galactose. It appeared that the barium salt of the aldobiuronic acid had been obtained in a more or less pure form.

Hydrolysis of the Barium Aldobiuronate.[1]

The barium aldobiuronate (0·25 g.) was hydrolysed, to effect a splitting of the molecule, with sulphuric acid (25 mls. of 2 N) for 25 hours at 100°.

$[\alpha]_D^{17°}$ Initial +60°, 1 hour +54°, 5 hours +46°,
10 hours +38°, 21½ hours +24°,
25 hours +22°.

Samples were withdrawn at the above-mentioned times and after neutralisation with barium carbonate were run on a strip chromatogram against standards. A gradual increase in the intensity of the spot corresponding to the standard L-arabinose was observed from 1 to 25 hours with an inverse change in intensity of the spot on the base line, due to the uronic and aldobiuronic acids.

A yellow barium salt (0·061 g.) was recovered on pouring into methanol (300 mls.) which had $[\alpha]_D^{17°}$ (c,0·48 in water) +20·6°; % CO_2, 17·8. The methyl-alcoholic solution was evaporated under reduced pressure to give a syrup (0·036 g.) which, on examination by strip chromatography, showed a strong spot corresponding to L-arabinose, with traces of L-rhamnose and D-galactose also present.

Oxidation with Bromine and Hydrobromic Acid.(2)

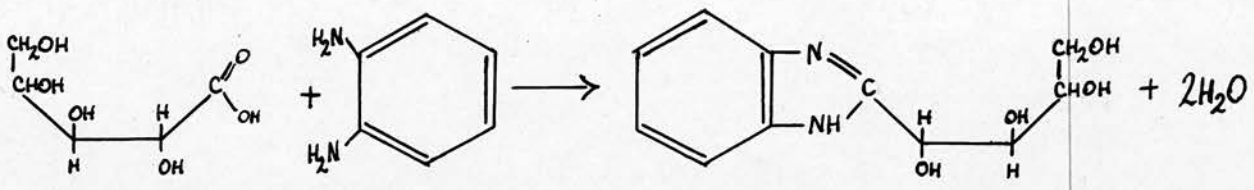
The barium aldobiuronate (0·50 g.) was dissolved in hydrobromic acid (6 mls. of 6%) containing bromine (0·25 mls.) and heated under reflux on a boiling water bath for 8 hours. The resulting product was filtered from a charred residue, and the filtrate allowed to stand in the refrigerator for 14 days when

a crystalline precipitate was obtained, filtered and the crystals dissolved in the calculated amount of dilute sodium hydroxide and reprecipitated by the addition of dilute hydrochloric acid. A white crystalline solid (needles) was obtained, m.p. 208°-9°. Mixed m.p. with an authentic sample of mucic acid was unchanged. The hydrobromic acid in the filtrate after removal of the crude mucic acid was neutralised by addition of silver carbonate, filtered, and the filtrate freed from Ag^+ by passage of hydrogen sulphide, filtered, and the aqueous solution evaporated under reduced pressure to give a yellow glass (0·381 g.) insoluble in methyl alcohol.

Formation of the Benzimidazole from the Aldonic Acid.[3]

To a test-tube containing the sugar acid (0·38 g.) was added o-phenylene diamine (0·30 g.), water (1 ml.), ethanol (0·25 ml.), concentrated hydrochloric acid (0·2 ml.) and syrupy phosphoric acid (0·2 ml.). The mixture with boiling chip was warmed until solution was complete and then heated for 2 hours on an oil bath at 135° ± 5°. Water boiled off during the condensation, leaving a thick syrup which was dissolved while warm in water (3 mls.) and filtered with carbon through asbestos. The filtrate was made basic with concentrated ammonia and the resulting precipitate washed well with water. A white solid was obtained which proved to be

inorganic in nature. The washings (8 mls.) were evaporated to dryness under reduced pressure, the resulting brown solid dissolved in a 50:50 mixture of alcohol and water (5 mls.), a little activated charcoal added, and the mixture refluxed for 1 hour. On filtration and complete removal of the solvent under reduced pressure, a white residue was obtained which, after washing carefully with water, alcohol and ether, had m.p. 231°-233° (with decomp.) 0·001 g. Mixed m.p. with an authentic sample of the benzimidazole from L-arabonic acid (m.p. 240°) was 234°-6°.



L-Arabonic Acid o-Phenylene Diamine Benzimidazole of L-arabonic Acid.

Methylation of the Aldobiuronic Acid.

The barium aldobiuronate (1·80 g.) was dissolved in water (20 mls.) and dimethyl sulphate (20 mls.) added dropwise along with sodium hydroxide (40 mls. of 30%) in 4 hours with vigorous stirring. After standing overnight, the reaction mixture did not reduce Fehling's solution. Solid sodium hydroxide (18 g.) was then added, followed by dimethyl sulphate (25 mls.) dropwise with stirring, during 7 hours.

When all the dimethyl sulphate had been added, the mixture was heated on the boiling water bath for 30 minutes, the solution cooled, acidified with cold concentrated sulphuric acid and extracted five times with chloroform. Evaporation of the chloroform extracts under reduced pressure left a syrup which was again methylated with dimethyl sulphate (20 mls.) and sodium hydroxide (40 mls. of 30%) as above, and then with solid sodium hydroxide (9 g.) and dimethyl sulphate (15 mls.). The product was isolated by chloroform extraction of the acidified solution as before, the syrup obtained being methylated three times with silver oxide (10 g.) added in six equal portions every hour, and methyl iodide (15 mls.). To ensure complete esterification, the product obtained from this methylation was treated with diazomethane in ethereal solution, the diazomethane removed by aeration after 24 hours at 0° and the resulting product distilled, a little barium carbonate being added to the distilling flask to combat any local acidity developing.

Two fractions were collected:-

(I) 115°-130°/0·05 mm. (bath temp.), 0·319 g., $\eta_D^{18°}$ 1·4503, OMe, 54·1%. Calculated for $C_{11}H_{20}O_7$ OMe, 58·7%.

(II) 170°-190°/0·05 mm. (bath temp.), 0·511 g., $\eta_D^{18°}$ 1·4687, OMe, 49·1%. Calculated for $C_{19}H_{34}O_{11}$ OMe, 49·5%; Calculated for $C_{18}H_{32}O_{11}$ OMe, 51·2%.

Investigation of Fraction I.

Fraction I (0·319) was hydrolysed with hydrochloric acid (15 mls. of 2 N) for 2 hours at 100°. $[\alpha]_D^{18°}$ Initial +14·4°, 1 hour +50·8°, 2 hours +50·8°. The solution was neutralised with silver carbonate, filtered, washed, and the volume reduced in vacuo to 25 mls. when liquid bromine was added (2 mls.) and the reactants kept at room temperature for 3 days, when on removal of the bromine by aeration the solution was found to be non-reducing to Fehling's solution. Neutralisation was accomplished by addition of barium carbonate and the filtrate and washings evaporated under reduced pressure, giving a white residue which was boiled with methyl-alcoholic hydrogen chloride (50 mls. of 3%) for 20 hours, at the end of which period the reactants were neutralised with silver carbonate and the filtrate evaporated under reduced pressure giving a white residue which was exhaustively extracted with boiling dry ether. Concentration of these extracts gave a yellow syrup which was distilled.

125°-35°/0·02 mm. (bath temp.), 0·169 g., $n_D^{16°}$ 1·4577. The last drop crystallised in the side arm and was added to the bulk to act as nuclei for further crystallisation. However, no further crystallisation occurred, and this fraction was not further investigated.

Investigation of Fraction II.

Fraction II (0·50 g.) was hydrolysed with hydrochloric acid (25 mls. of 2 N) for 10½ hours at 100°. $[\alpha]_D^{17°}$ Initial +121°, 1 hour +116°, 3 hours +112°, 6 hours +95°, 10½ hours +82°. The hydrochloric acid was neutralised with silver carbonate, filtered, washed, and the Ag^+ removed by the passage of hydrogen sulphide, filtered, and the excess hydrogen sulphide removed by aeration. The volume was reduced in vacuo to 25 mls., and barium carbonate added, the filtrate evaporated under reduced pressure to give a syrup which was exhaustively extracted with dry boiling ether, when a syrup A (0·121 g.) and a residue B (0·400 g.) were obtained.

The residue B was dissolved in water (10 mls.) and liquid bromine (1 ml.) added, the reaction kept at room temperature for 4 days, and finally at 50° for 3 hours, when on removal of the bromine by aeration the reducing action to Fehling's solution was found to have disappeared. Barium carbonate was added to effect neutralisation, filtered, washed, and the filtrate evaporated to dryness under reduced pressure to give a white residue which was refluxed with methyl-alcoholic hydrogen chloride (25 mls. of 3%) for 23 hours. Neutralisation was effected by addition of silver carbonate and the filtrate evaporated under reduced pressure, giving a white residue which was exhaustively

extracted with dry boiling ether. Concentration of these extracts gave a yellow syrup which was distilled. 120°-35°/0·01 mm. (bath temp.), 0·140 g., last drop $n_D^{19°}$ 1·4580, OMe, 56·1%.

The residue was extracted from the flask with acetone and charcoaled to give, on removal of the solvent under reduced pressure, a yellow syrup (0·071 g.), $n_D^{19°}$ 1·4780.

Partial crystallisation occurred in the distillate, the crystals recovered by tiling and on recrystallisation from light petroleum (40°-60°) had m.p. 94°-8° (0·005 g.). Mixed m.p. with an authentic sample of the dimethyl ester of 2:3:4-trimethyl mucic acid (m.p. 99-100°), was 94°-7°.

No value for the specific rotation of Syrup A could be obtained, owing to the formation of a fine suspension in aqueous solution, a sample of which was run on a strip chromatogram against standards and on development showed two spots of almost equal intensity of R.G. values 0·543 and 0·769. No reference compounds of R.G. values approximating to the above could be found in the literature.(4) To the remainder of the aqueous solution (5 mls.) liquid bromine (1 ml.) was added, and the reactants kept at room temperature for 4 days, when on removal of the bromine by aeration the reducing action to Fehling's solution was found to have disappeared. The lactone was prepared as previously

described, and on distillation gave a yellow syrup.

115°-30°/0·01 mm. (bath temp.), 0·049 g., last drop $n_D^{19°}$ 1·4635.

$[\alpha]_D^{16°}$ (c, 1·00 in water):

Initial +20°, 3 hours +10°, 8 hours +2° (constant value).

The amide was prepared as usual, and on removal of the methyl-alcoholic ammonia in a vacuum desiccator, a white solid was obtained which on recrystallisation from ethyl acetate by addition of light petroleum (40°-60°) had m.p. 147°-8°, (0·015 g.). Mixed m.p. with an authentic sample of 3:4-dimethyl-L-rhamnonamide (m.p. 144°-7°) was 110°-126°. Mixed m.p. with an authentic sample of 3:5-dimethyl-L-arabonamide (m.p. 145°) was 117°-25°.

(Found: C, 43·0; H, 7·85; N, 7·08 ; OMe, 28·2. $C_7H_{15}O_5N$ requires C, 43·5 ; H, 7·7 ; N, 7·25 ; OMe, 32·1; and $C_8H_{17}O_5N$ requires C, 46·4 ; H, 8·2 ; N, 6·76; OMe, 30·0%).

DISCUSSION Of RESULTS.

3% Oxalic acid was used in hydrolysis of the "free acid" polysaccharide when an insoluble residue (9·8%) was obtained, which was probably cellulosic in nature,[5] and a barium salt (8·8%) which was rehydrolysed with 3% oxalic acid and again precipitated (5·9%) having % CO_2, 6·9. $(C_{12}H_{21}O_{11})_2Ba$ requires % CO_2 , 10·7 ; % Ba, 16·7 . From the value obtained for the percentage CO_2 it appeared that more than one sugar residue was still attached to the uronic acid unit and that more efficient hydrolysis would be required to obtain the aldobiuronic acid. 4% Sulphuric acid was therefore employed for rehydrolysis, and the barium salt (3·4%) recovered showed % CO_2 , 7·3 ; % Ba, 10·1. The sugars split off as a result of hydrolysis with 4% sulphuric acid were examined by strip chromatography[4] and shown to be a mixture of L-arabinose, D-xylose and L-rhamnose with a trace of D-galactose, the presence of L-arabinose being confirmed by the preparation of L-arabinose diphenylhydrazone, while the percentage of L-arabinose in the mixture was calculated to be 35·4. Partial crystallisation of the L-rhamnose monohydrate occurred after allowing the syrup to stand for several weeks. Hydrolysis of the barium salt was continued

using 6% sulphuric acid and the barium salt precipitated (2·3%) had % CO_2 , 9·4 ; % Ba, 15·4 ; $[\alpha]_D$ +49·7°. The syrupy hydrolysate, on examination as before, showed the presence of L-arabinose only with traces of D-xylose and D-galactose. The percentages of carbon dioxide and barium found coupled with the results of the examination by strip chromatography seemed to indicate that a barium aldobiuronate had at last been obtained in reasonably pure form.

A portion of the barium aldobiuronate was hydrolysed with 2 N(9·8%) sulphuric acid for 25 hours. $[\alpha]_D$ initial +60° to +22° (25 hours). The hydrolysis was followed by strip chromatography and the spot due to L-arabinose was found to increase gradually in intensity as hydrolysis progressed. The barium salt of the uronic acid recovered showed $[\alpha]_D$ +20·6°, and % CO_2 , 17·8. The syrupy mixture of sugars consisted mainly of L-arabinose with traces of L-rhamnose and D-galactose.

Oxidation with bromine and hydrobromic acid[2] for 8 hours resulted in the isolation of a small amount of mucic acid (m.p. 208°-9°) and formation of the benzimidazole[3] gave a small quantity of the benzimidazole of L-arabonic acid (m.p. 231°-3°). It appeared from the results of the hydrolysis and oxidation carried out on the barium aldobiuronate that the two main residues involved in the formation of the

aldobiuronic acid were D-galacturonic acid and L-arabinose.

A portion of the barium aldobiuronate was methylated with sodium hydroxide and dimethyl sulphate, followed by three methylations with silver oxide and methyl iodide and finally with diazomethane to ensure esterification of the carboxylic grouping. Distillation yielded two fractions: (I) 115°-30°/0·05 mm. (bath temp.), 0·319 g., $\eta_D^{18°}$ 1·4503, OMe, 54·1%. (II) 170°-90°/0·05 mm. (bath temp.), 0·511 g., $\eta_D^{18°}$ 1·4687, OMe, 49·1%.

Fraction (I) appeared to be the methyl ester of the methylated uronic acid, and from the quantity present (62%) it seemed that hydrolysis of the barium salt with 6% sulphuric acid had been somewhat drastic. Hydrolysis of the ester with 2 N hydrochloric acid for 2 hours $[\alpha]_D$ +14·4° to +50·8° (2 hours), followed by oxidation with bromine to the dicarboxylic acid and subsequent esterification with 3% methyl-alcoholic hydrogen chloride gave a yellow syrup which was distilled, 125°-35°/0·02 mm. (bath temp.), 0·169 g., $\eta_D^{16°}$ 1·4577. This fraction did not crystallise and was not further investigated.

Fraction (II) was hydrolysed with 2 N hydrochloric acid for 10½ hours $[\alpha]_D$ Initial +121° to +82° (10½ hours), and the resulting syrup exhaustively extracted with boiling ether to give a syrupy fraction A

and a residual B containing the partially methylated uronic acid. The corresponding dicarboxylic acid was prepared from the methylated uronic acid by oxidation with bromine and the diester then formed by refluxing for 23 hours with 3% methyl-alcoholic hydrogen chloride.

Distillation of the diester gave: 120°-35°/0·01 mm. (bath temp.), 0·140 g., last drop $n_D^{19°}$ 1·4580, OMe, 56·1%. The undistilled residue (0·071 g.) $n_D^{19°}$ 1·4780 probably resulted from incomplete hydrolysis of the methylated aldobiuronic acid and would be present at this stage as the dimethyl ester of a combined aldonic and uronic acid.

The distillate crystallised partly on standing, and the crystals recovered (m.p. 96°-8°) showed no significant depression of the m.p. (94°-7°) when admixed with an authentic sample of the dimethyl ester of 2:3:4-trimethyl mucic acid (m.p. 99°-100°), thus confirming the presence of D-galacturonic acid in the aldobiuronic acid linked through C_1.

Examination of syrup A by strip chromatography showed it to be a mixture of two sugars, R.G. values 0·54 and 0·77, and from the relative intensity of the spots appeared to be present in almost equal concentrations. The sugar of R.G. value 0·54 possibly corresponded to a dimethyl pentose, and that of R.G. value 0·77 to a dimethyl methylpentose, but no reference

compounds were available of R.G. values approximating to the above, nor was the limited publication on the subject[4] of much assistance in identification. Lactone formation gave a syrup which on distillation yielded:

115°-30°/0·01 mm. (bath temp.), 0·049 g. last drop $\eta_D^{19°}$ 1·4635.

$[\alpha]_D$ Initial +20 to +2 (8 hours): that is, this appeared to be a mixture of δ-lactones. A crystalline amide was obtained (0·015 g.), m.p. 147°-8°, which was identical with neither 3:4-dimethyl-L-rhamnonamide nor 3:5-dimethyl-L-arabonamide. Analysis of the amide identified it as a dimethyl pentose amide, while investigation of the unmethylated aldobiuronic acid (p.160) suggested an arabinose derivative, but lack of material did not allow of further investigation.

The dimethyl arabonolactones which form δ-lactones are the 2:3, 2:4 and 3:4-dimethyl arabonolactones. The 2:3-dimethyl arabonamide has m.p. 162° and the 2:4-dimethyl arabonamide, m.p. 158°, neither of which are in agreement with the value found (147-8°). There remains the possibility that the methylated aldobiuronic acid contained the hitherto unprepared 3:4-dimethyl arabopyranoside, linked through C_2. However, further investigation will be required to elucidate this problem.

S U M M A R Y.

1. Hydrolysis of the "free acid" polysaccharide was carried out with 3% oxalic acid (20 hours) and the crude barium aldobiuronate so obtained was further hydrolysed with 3% oxalic acid (6 hours), 4% sulphuric acid (6 hours) and 6% sulphuric acid (6 hours). A large proportion of L-arabinose and L-rhamnose was detected as a result of these hydrolyses with traces of D-xylose and D-galactose.

2. Hydrolysis of the barium aldobiuronate with 9·8% sulphuric acid (25 hours) was accompanied by a gradual increase in the concentration of L-arabinose, while traces of L-rhamnose and D-galactose were also present. The barium salt of the uronic acid had $[\alpha]_D$ +20·6° ; % CO_2 , 17·8.

3. Oxidation of the barium aldobiuronate with bromine and hydrobromic acid resulted in the isolation of mucic acid, from D-galacturonic acid, and identification of L-arabinose as the benzimidazole of L-arabonic acid.

4. Methylation of the barium aldobiuronate was carried out with sodium hydroxide and dimethyl sulphate, silver oxide and methyl iodide, and finally with diazomethane. On distillation, two fractions were obtained, (I) being

a methylated uronic acid ester which was converted to the diester of the dicarboxylic acid; however, no crystallisation occurred in this fraction and it was not further investigated. (II) was hydrolysed with 2 N hydrochloric acid (10½ hours) and the uronic acid portion identified as D-galacturonic acid, linked through C_1 by the isolation of the dimethyl ester of 2:3:4-trimethyl mucic acid. This fraction also yielded a mixture of two partially-methylated sugars; the crystalline amide prepared from one (m.p. 147°-8°) appeared from analysis data to be a dimethyl pentose amide, but lack of material prevented further investigation.

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